THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. XXXIX—NO. 4

Issued February 1, 1916

BALTIMORE, U. S. A.

1916

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879

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WEST WASHINGTON SQUARE
PHILADELPHIA, U. S. A.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 39 FEBRUARY 1, 1916 No. 4

THE INFLUENCE OF HEAVY METALS ON THE ISOLATED INTESTINE

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FIRST COMMUNICATION

Received for publication December, 1, 1915

The pharmacology of the isolated intestine has been made the subject of a number of investigations within recent years. Kuliabko and Alexandrowitsch (1) were the first to test the action of different drugs by this method. A similar investigation was carried out later by Magnus (2) on the intestine of the cat, while Kress (3) studied the action of the same drugs on the intestine of the dog and of the rabbit. Sembdner's (4) experiments with chloral, Kuno's (5) work with the alcohols, the studies on the action of members of the fatty acid series made by Rona and Neukirch (6), Starkenstein's (7) studies with calcium precipitants and the recent communications of Hanzlik (8) on chelidonin may also be mentioned in this connection. The tests on pilocarpine made by Neukirch (9) mark the first attempt at quantitative pharmacological studies on isolated segments of intestine. This method was also employed by Kuyer and Wijsenbeck (10) for the investigation of the antagonistic action of drugs.

The influence of the heavy metals on the intestine has received very little attention as yet, Siccardi's (11) experiments with lead acetate on the intestine of the rabbit being the only communication on the subject we could find in the literature. The present report aims at presenting some results obtained with zinc, in the form of malate, and nickel, of which the acetate was used, on different parts of the small intestine and colon of the cat and the rabbit.

Studies were also made on the reaction to barium chloride, pilocarpine and atropine after being subjected to the influence of the heavy metals. The method devised by Magnus and carried out in this laboratory as described in a recent publication by us (12) was also used in the present investigation. The zinc, as well as the nickel salt, was dissolved and added to Locke solution which was maintained at a temperature of 37 to 38° C.

The action of zinc. The toxicity of zinc has been established by experiments on lower organisms, as well as on higher animals. According

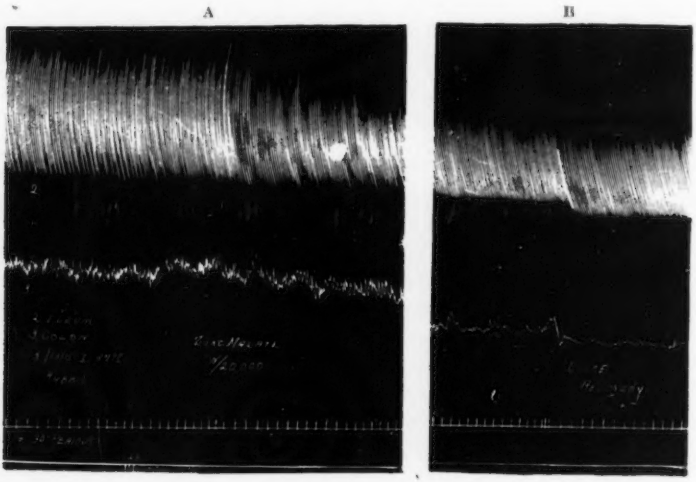

Fig. 1. Rabbit 1789. A, in zinc malate N/20,000, 15 minutes. B, in zinc malate at the end of 43 to 60 minutes. Also shows contractility in Locke solution alone after previous treatment.

to Freitag (13) its presence in nutrient solutions in a concentration of 0.02 per cent kills the roots of some phenoragamic plants. More recently Hawkins (14) has obtained similar results in experiments on algae. According to Harnack (15) the administration of zinc salts to higher animals causes symptoms of depression and paralysis of the muscles, respiration and circulation.

Experiments on the isolated intestine of the rabbit. Segments of the small intestine and of the colon manifested signs of decreased activity soon after they were suspended in a dilute solution of zinc salt. Very

low concentrations, N/20,000 (fig. 1) and in one case N/30,000, caused a well marked depression. The concentration was gradually increased, but a marked difference in the results was first observed when a solution of N/10,000 was tried. After a brief period of stimulation involving tonus and rhythmic action, depression set in and continued steadily 15 to 60 minutes; the contractions then remained uniform but much reduced in size (figs. 2, 3). Occasionally decreased frequency of action could also be observed at this stage. The course of events varied somewhat in different parts of the intestine. Rise of

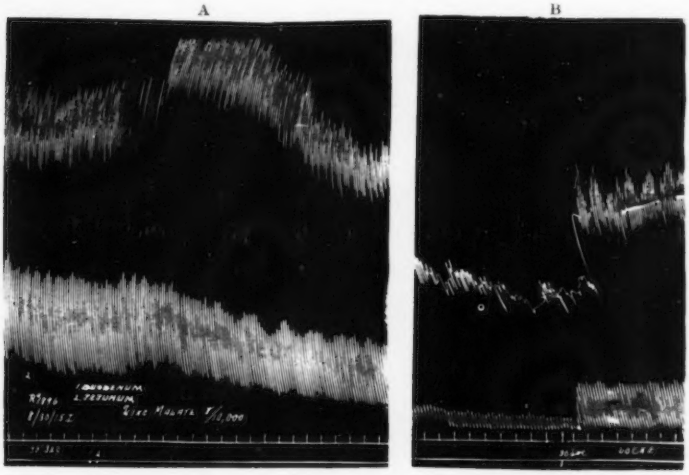

Fig. 2. Rabbit 1896. Zinc malate N/10,000. A, after 10 minutes. B, after 35 to 45 minutes and when changed to pure Locke solution.

tonus and irregularity were more frequent in the duodenum; decrease of tonus was sometimes noticed in the colon, but such changes seldom occurred in the jejunum and ileum. When suspended in pure Locke solution again, after thoroughly washing, considerable improvement was observed even when contact with zinc malate lasted 70 minutes. Complete recovery, however, never occurred although the action of zinc was in some cases limited to a period of 45 minutes only. That the tissues were permanently damaged also appeared in experiments, which showed the effect of several treatments with the salt. The preliminary stimulation was absent while depression set in almost imme-

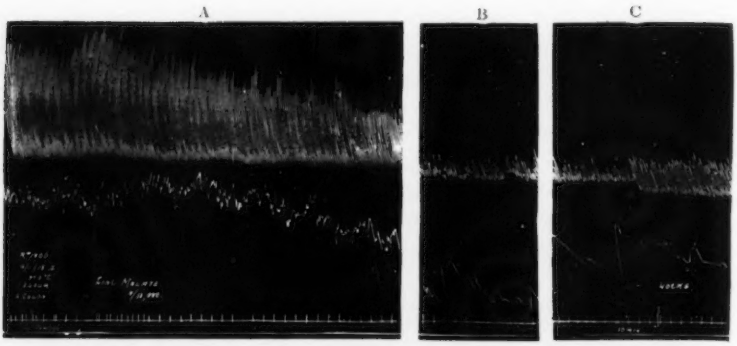

Fig. 3. Rabbit 1900. Zinc malate N/10,000. A, shows initial stimulation followed by depression of ileum and colon. B, contractions of the ileum much reduced in size at the end of 46 to 52 minutes in zinc malate; tonus decreased in colon. C, moderate improvement when returned to Locke solution shown, amplitude of rhythmic contractions and tonus of ileum increased when changed to pure Locke solution but did not recover.

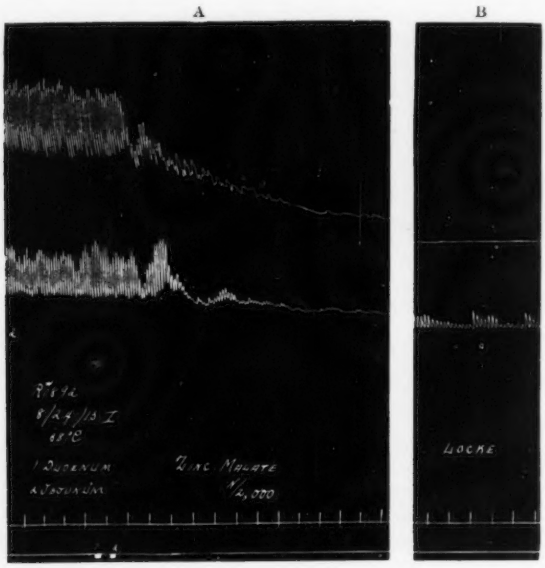

Fig. 4. Rabbit 1892. Duodenum and jejunum. Zinc malate N/2000. A, marked depression. B, shows extent of recovery when returned to Locke solution alone. Segments subjected to zinc malate for 30 minutes.

diately after the zinc salt was added, the progressive decrease of amplitude being much faster than in experiments in which the intestine was subjected to the action of zinc for the first time. Contractility was abolished within 28 minutes in the latter case while the same effect was produced in a few minutes after the second treatment with zinc salt. A moderate amount of decrease in the rate of contraction was also observed. When suspended again in pure Locke's solution the improvement noticed was slight in one segment while contractions were absent in another, although it was exposed to the action of zinc salt for a period of about 30 minutes only.

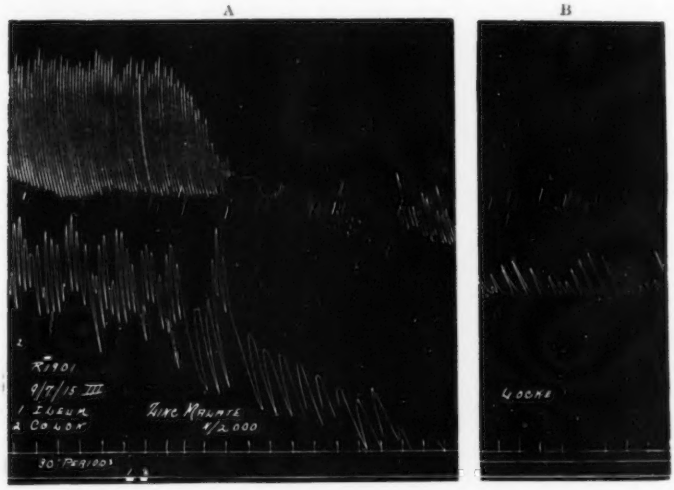

Fig. 5. Rabbit 1901. Ileum and colon. A, effect of zinc malate N/2000. B, recovery in Locke solution after subjection to zinc malate for 45 minutes.

The action of N/5,000 zinc malate was more marked than in experiments with N/10,000, the difference observed being appreciable. The preliminary rise, however, could still be noticed in some instances. Depression of rhythmic contractions and of tonus set in promptly upon the addition of the salt and reached a maximum within 6–20 minutes after the zinc malate was added. The contractions disappeared or became very weak at this time. Irregular action also appeared. Recovery when placed in Locke's solution after exposure to the action of the zinc salt for 40 to 45 minutes was incomplete. Only slight

improvement in contractility was observed in some experiments in the duodenum and jejunum. The contractions in the ileum were pronounced but were less forcible than in the fore period.

The results obtained in experiments with higher concentrations indicate that the activity of zinc malate was considerably greater but the difference was not in proportion to the amount of the salt present in solution. Depression without initial stimulation was observed with

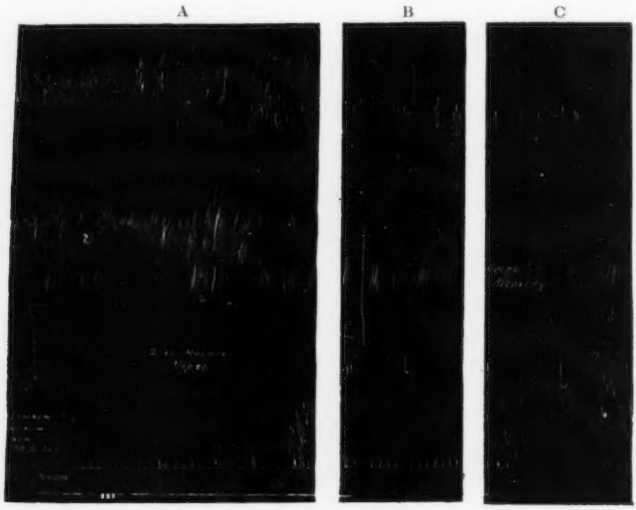

Fig. 6. Cat 370. Zinc malate N/5000. Duodenum, jejunum and ileum; upper, middle and lower tracings. A, primary stimulation of duodenum and jejunum with depression of ileum. B, 46 to 54 minutes in zinc malate. Frequency of rhythmic contractions decreased in duodenum, abolished in jejunum. Ileum shows improvement. C, the effect of pure Locke solution. Duodenum. Contractions less frequent than before. Jejunum. No contractions. Ileum shows stimulation.

a solution of N/2000 (figs. 4 and 5) after 1.5 to 2 minutes in some experiments; in others this occurred in about 30 seconds after the zinc malate was added. Approximately 4 minutes usually elapsed before contractions ceased in the duodenum and jejunum, but the suppression of activity was delayed considerably in the ileum. Rhythmic contractions, though feeble, persisted 20 to 25 minutes in some experiments after zinc malate was added. Eleven minutes was the shortest period

of activity of the ileum as a result of treatment with N/2000 solution. After being in contact with malate 17 to 45 minutes and then placed in Locke's solution, contractility was slight, or absent in the duodenum and jejunum but was distinct and in some cases well marked in the ileum, thus showing once more the greater resistance of this portion of the intestine to zinc malate. When the concentration was increased to N/1000 pronounced depression was observed almost immediately, or within half a minute after adding the salt. The contractions ceased in two to five minutes. Depression of tonus was very marked in some sections of the intestine, but was absent in others. When suspended in Locke's solution alone very feeble contractions, or none at all, were observed in the duodenum and jejunum, even when previous treatment with zinc malate was only eight minutes, but the improvement in the ileum was constant. Tests were also carried out with N/500 zinc malate. Marked depression of tonus and complete cessation of rhythmic activity set in promptly after the addition of salt.

Experiments on the intestine of the cat. The results obtained show that the response to zinc is less marked in the intestine of these animals than in that of the rabbit. (See figs. 6 and 7). A solution of N/5,000 zinc malate produced depression in the duodenum and jejunum in about 25 minutes which proceeded gradually to complete extinction of the contractions about a half hour later. In nearly every case this followed stimulation which was preceded sometimes by initial depression occurring promptly after the addition of the salt. Although the activity of the ileum was also decreased, complete inhibition of contractility was never observed. That this portion of the intestine is more resistant to zinc was also shown by its recovery when it was returned to Locke's solution, whereas neither the duodenum nor the jejunum showed any signs of improvement when subjected to the same treatment.

In a series of experiments with N/2,000 zinc malate two types of response could be distinguished. In one, gradual depression set in and continued steadily until all contractility disappeared within 18 to 25 minutes, sometimes within 8 minutes. In the other type, depression set in promptly and contractions disappeared, but returned at the end of 6 to 16 minutes. When placed in Locke's solution, improvement occurred in only one experiment on the ileum. A noticeable difference in the behavior of the intestine was observed when it was treated with stronger solutions. Contractions disappeared promptly in N/500 and in 6 to 10 minutes in N/1,000 zinc malate. In one experiment,

however, activity continued 28 minutes. A return of contractility was never observed even when Locke's solution was substituted. It may be remarked that the ileum showed greater resistance also in these experiments as it continued its activity in one case 10 minutes in N/500 solution and 45 minutes in N/1,000, though the strength of

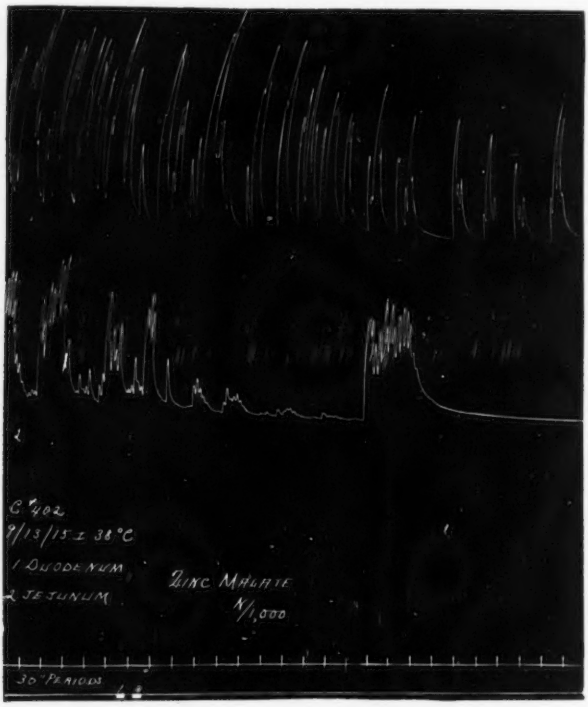

Fig. 7. Cat 402. Note contractions of duodenum after 10 minutes' exposure to N/1000 zinc malate.

the contractions was considerably reduced. It might be added that in this case weak but distinct contractions were observed when pure Locke's solution was substituted for one containing zinc salt.

Reaction to pilocarpine, barium and atropine. The tests were carried out on rabbit's intestine in the presence of zinc salt, these substance being added to the solution at various intervals, thus permitting

of the study of the influence of time on the reaction of the intestine. Absence of the normal response to pilocarpine could be observed shortly after the intestine was subjected to the influence of a weak solution of zinc malate. Pilocarpine hydrochloride, 1 : 200,000, produced only moderate stimulation in the jejunum and ileum 4½ minutes after these segments had been subjected to the influence of N/5,000 zinc malate. In another experiment the reaction to pilocarpine tested after the intestine had been subjected to N/5,000 zinc malate for 22 minutes caused but a slight response. A reaction to pilocarpine could still be obtained when higher concentrations, N/2,000 and N/1,000 of zinc malate were used; fifteen minutes after the segments of the jejunum and ileum had been suspended in N/2,000 zinc malate and contractions had disappeared, a slight reaction to pilocarpine was produced. The same concentration of the alkaloid was still effective in the presence of N/1,000 zinc malate after 15 minutes. A slight response was observed in the ileum in another experiment in which the test was made after 21 minutes, but no reaction to pilocarpine was observed in a third experiment with N/1,000 zinc malate. It might be added that the intestinal segments were in a state of relaxation or paralysis in all experiments with N/2,000 and N/1,000 and at the time pilocarpine was tested. Only the jejunum was in this condition after 22 minutes in N/5,000 zinc malate. Experiments in which pilocarpine preceded treatment with zinc salt were also performed. The stimulation produced by the alkaloid was promptly antagonized by a solution of N/5,000 of the salt, and the activity of the intestine gradually decreased as in the experiments with zinc alone.

The reaction of the zinc treated intestine to barium chloride was preserved considerably longer and was much more pronounced than the response to pilocarpine (see fig. 8). Some time after spontaneous contractions disappeared and no reaction to pilocarpine could be obtained, very marked stimulation with barium chloride could be induced. Thus in two experiments with N/5,000 zinc malate, a response was obtained in one case 17 minutes after all contractions ceased; in another experiment a reaction was observed in a paralyzed segment of the jejunum which had been acted upon by zinc for 42 minutes. When treated with solutions of N/2,000 and N/1,000 zinc malate similar results were obtained. The addition of barium chloride after 30 minutes exposure of the intestine to zinc salt was followed by the appearance of contractions. Spontaneous movements before this test was made were absent. Attempts to obtain a reaction with pilocarpine were

unsuccessful. The reaction to atropine in the presence of zinc was likewise tested in three experiments. It may be recalled that Kress has shown that small amounts of atropine stimulate intestinal contractility. No effect was obtained in two experiments, and well marked depression was produced in the third, N/5,000 and N/2,000 zinc malate being used in these tests.

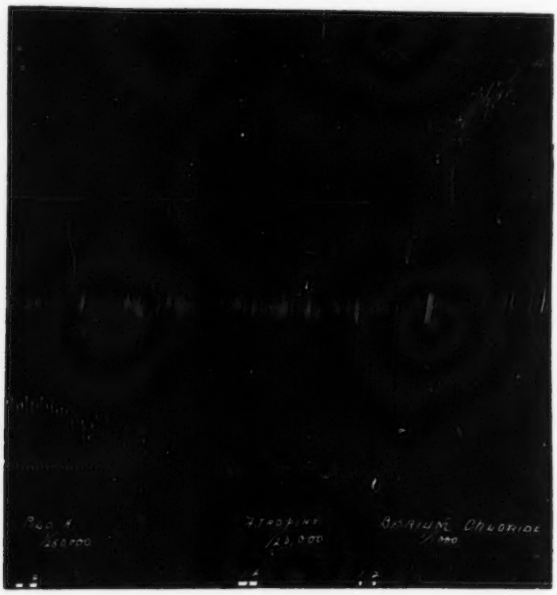

Fig. 8. Rabbit 1905. In N/2000 zinc malate four minutes. Upper tracing, jejunum; lower, ielum. Neither pilocarpine hydrochloride 1: 250,000 nor atropine sulphate had any effect but barium chloride caused prompt and very pronounced contractions.

The following two protocols are typical of the experiments with the reaction to pilocarpine, barium and atropin:

R. 1904. One minute after jejunum and ileum were suspended in N/5,000 solution of zinc malate marked depression and irregularity were observed, the contractions being very weak in the former. Twenty two minutes later 1 : 200,000 pilocarpine hydrochloride produced slight stimulation in the jejunum. Very weak contractions appeared at inter-

vals of about a minute or more. The effect on the ileum was greater, though less than on the normal intestine. Eight minutes later the same amount of pilocarpine was added again, the reaction was slightly greater in the jejunum but was weaker in the ileum than in the previous test. At the end of 50 minutes exposure to zinc malate the jejunum ceased to contract but the ileum was still active. Barium chloride 1/1,000 produced a well marked reaction which was much greater in the ileum than in the jejunum.

R. 1905. The effect of pilocarpine in the proportion of 1 : 250,000 was tested four minutes after the segments of the jejunum and ileum had been exposed to the action of N/2,000 zinc malate. The contractions of the ileum which were fairly strong before the alkaloid was added to the Locke's solution steadily decreased until they became very weak and irregular thirty minutes later, while the jejunum remained inactive as before the pilocarpine was introduced into the solution. Barium chloride which was added five minutes after pilocarpine produced a powerful contraction in the ileum, the tonus remaining very high for a considerable length of time, rhythmic contractions also appeared. The jejunum likewise shared in this stimulation but to a much smaller extent. Neither segment was influenced by the previous addition of atropine sulphate.

The action of nickel. The effects of nickel on different animals has been studied by Gehrkens (17), Stuart (18) and Bulatow (19). Severe symptoms and death were observed after the subcutaneous and intravenous administration of small doses of nickel salts. According to Stuart and to Bulatow a fall of blood pressure is also caused when the salt is injected intravenously. That it is toxic to lower organisms appears from the observations of Hawkins (20) who studied the behavior of fungus spores toward nickel nitrate.

Experiments on the intestine of the cat. Various concentrations were employed in these tests. Solutions of N/50,000 and N/20,000 failed to produce any demonstrable change although allowed to remain in contact with the tissues for a considerable period of time. A distinct after effect could be observed, however, in a few cases in which total suppression of rhythmic contractions occurred when the solution of nickel acetate was replaced by pure Locke solution. Definite results, though not very pronounced, were obtained with a solution of N/10,000. Depression and sometimes complete abolition of rhythmic action with decreased tonus were the first manifestations of a reaction to the metal and lasted 25 to 35 seconds. This was followed, however, by a period

of progressive improvement ending in recovery in the jejunum while in the ileum tonus as well as rhythmic contractions became even greater than in the fore period (figs. 9 and 10). In neither case was the sub-

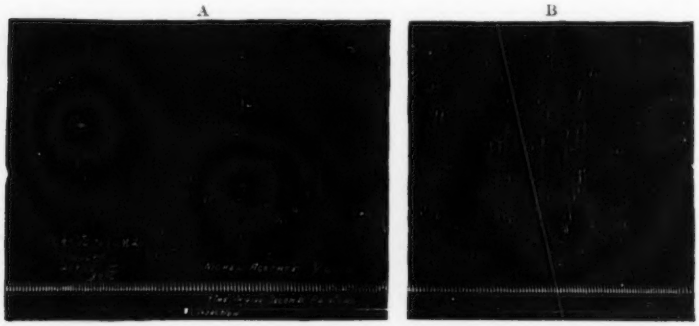

Fig. 9.

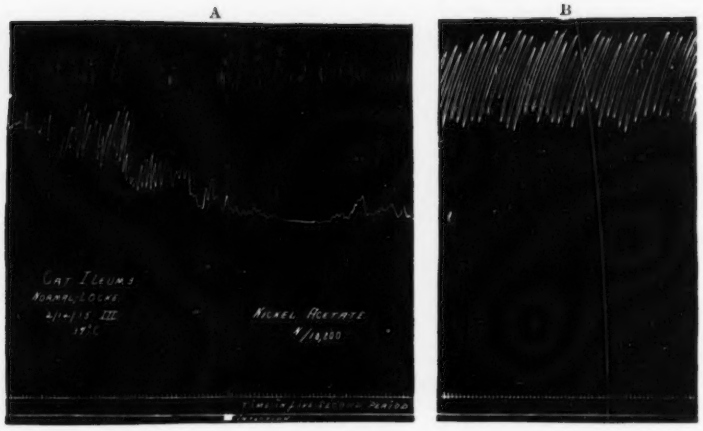

Fig. 10.

Fig. 9. Cat 328. A, primary depression. B, contractions after 25 minutes in nickel acetate.

Fig. 10. Cat 331. A, primary depression. B, stimulation after one hour in N/10,000 nickel acetate.

stitution of pure Locke solution followed by any noteworthy changes. A few experiments were also carried out with N/5,000 nickel acetate. Depression of tonus with suppression of contractions were present as

in experiments with N/10,000 nickel acetate, but recovery was delayed longer than in the latter. Prompt and total inhibition of muscular contraction with decrease of tonus was more frequent and the effect more lasting with higher concentrations. Segments of the jejunum and ileum which were treated with N/2,000 nickel acetate ceased their activity almost immediately. They were under observation 12 to 18 minutes, but no change could be noticed. The effect on the colon was less marked. Complete abolition of contractions was exceptional. In most experiments moderate depression was the only effect produced in the colon. This also occurred with a solution of N/1,000 shown in Cat 386 (fig. 11) in which pronounced depression of the colon was soon

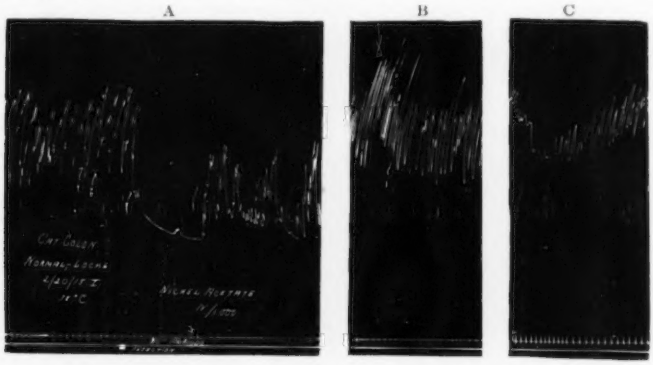

Fig. 11. Cat 386. Colon in nickel acetate N/1000. A, depression of tonus and rhythmic contractions. B, condition of intestine 1¾ hours after adding nickel acetate. C, condition in pure Locke solution.

followed by improvement ending in recovery while still in nickel acetate. In another experiment on the colon with the same concentration, the primary depression was followed by recovery and stimulation while still in nickel acetate. The injury caused by nickel acetate is not permanent, however, as shown in experiments with the higher concentrations. It was noticed that when pure Locke solution was substituted for N/2,000, N/1,000 and N/500 nickel acetate, improvement and sometimes complete recovery took place although it had been acted upon by the metal for periods of time varying between 25 and 54 minutes.

The action of nickel on the intestine of the rabbit. Disturbance of muscular action in the intestine of the rabbit was observed even when

low concentrations of nickel acetate were employed. A solution of N/10,000 nickel acetate already produced marked effects causing at first depression then stimulation of tonus accompanied by a decrease and irregularity of amplitude of the rhythmic contractions. It occurred almost immediately after the metal was added to Locke solution and lasted 2 to 6 minutes. This was succeeded by gradual increase in the force of contractions, the amplitude becoming appreciably greater than in the fore period, but the rate remained unchanged. Within 12

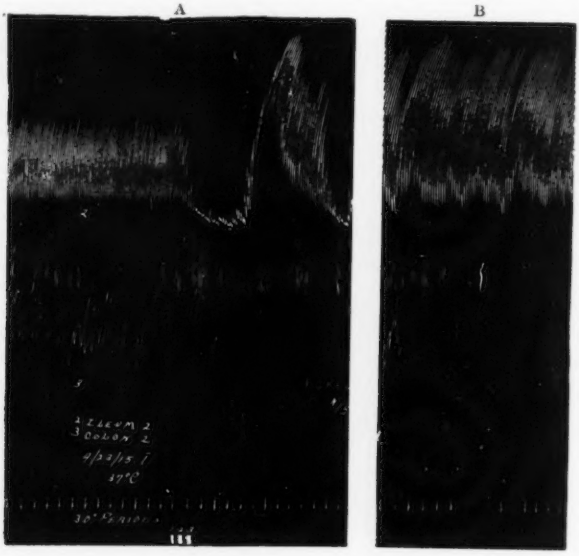

Fig. 12. Rabbit 1803. Ileum and colon. A, segments subjected to nickel acetate N/5000. B, 45 minutes after addition of nickel acetate.

to 18 minutes after the test with the metal was begun the progressive rise of the amplitude ceased and remained uniform to the end of the experiment. Although the results obtained were practically the same in most cases, a few exceptions were noted. The addition of nickel acetate was followed in some tests on the jejunum by gradual depression without recovery. The action of N/5,000 nickel acetate differed but little from that caused by a solution of N/10,000. Depression of rhythmic contractions was followed by gradual recovery in the presence of the salt (fig. 12). In some experiments dis-

tinct stimulation was noticed, the contractions becoming stronger than in the preliminary period. Temporary disturbance of tonus was likewise observed. Most of the experiments were carried out on the jejunum, but tests were also performed on the duodenum and ileum. When pure Locke solution was substituted for one containing nickel acetate, marked decrease of rhythmic contractility was observed after a solution of N/10,000 as well as after N/5,000. The depressive action of more concentrated solutions was more pronounced. N/2,000 nickel acetate produced in the various sections of the small intestine and in the colon a marked decrease of the size of the rhythmic contractions, frequency not being affected. Marked decrease of tonus was also noticed. Gradual improvement while still in the salt solution took place, but the maximum strength obtained was below that in the fore period. In one case only was stimulation with considerable increase of amplitude observed. A marked fall of tonus occurred in the duodenum and lasted 3 to 5 minutes but recovered gradually.

A solution of N/1,000 nickel acetate promptly abolished rhythmic contractions, tonus also being markedly depressed at the same time in nearly all experiments. Improvement was observed in all experiments with this concentration but it was delayed considerably. Contractions began to return in some cases after 7 to 9 minutes while sometimes it was delayed 22 minutes. It may be remarked in this connection that similar effects although much less frequent were observed with much more concentrated solutions. Segments of the jejunum and ileum suspended in N/500 nickel acetate ceased to contract promptly upon the addition of the salt. During a period of observation lasting 17 minutes no change was observed in the jejunum, but contractions returned in the ileum and became appreciable in 3 minutes.

Reaction to pilocarpine and barium. That the intestine preserved its irritability after nickel acetate was also shown by tests with pilocarpine and barium chloride. Although contractions were abolished when the concentrations used were sufficiently high the addition of pilocarpine promptly restored muscular activity. Powerful contractions appeared in segments of the intestine previously made inactive by a solution of N/1,000 nickel acetate. The effect also showed a tendency to continue over a considerable period of time, no abatement of the stimulating action of pilocarpine could be noticed during periods of observation of 16 and 28 minutes. The effect varied with N/500 nickel acetate, the reaction to pilocarpine being very pro-

nounced in some experiments, while a weak reaction only was observed in another experiment; tests in both cases were made on jejunum and ileum. Failure to react to pilocarpine was first observed in some experiments with N/200, while no reaction could be noticed after N/100 was used. Barium chloride when added in sufficient amount, however, produced a distinct response. Tonus and rhythmic contractions reappeared. The stimulating effect of barium was also noticed in a number of experiments in which no reaction to pilocarpine could be obtained. Thus in one experiment on the jejunum and ileum a rise of tonus only occurred in the jejunum, and rhythmic contractions of moderate strength were observed in the ileum when barium chloride 1/500 was added 25 minutes after the intestine had been suspended in N/100 nickel acetate. Pilocarpine hydrochloride tried 10 minutes before barium had no effect. Similar results were obtained with eserine and barium chloride on the intestine of the cat which had been subjected to the action of N/1,000 nickel acetate. Barium chloride 1:500 which was added 12 minutes after nickel acetate, stimulated the duodenum, jejunum and ileum so that the contractions became considerably stronger than in the fore period while eserine in the proportion of 1:100,000 had no effect.

DISCUSSION

Although depression may be produced by the more concentrated solutions of either metal, a marked difference in their effect followed exposure of the intestine to dilute concentrations of zinc or nickel. In experiments on the rabbit's intestine, a solution of N/10,000 zinc malate caused depression of tonus and decrease of amplitude within one-half to 3 minutes. At the end of three-quarters to one hour, and sometimes after 15 minutes only, rhythmic contractions were reduced to a small portion of their original size. The same concentration of nickel acetate sometimes caused primary depression for a brief period but was usually followed by recovery and stimulation. This was also observed in experiments with a solution of N/5,000 nickel acetate. That the toxicity of zinc is greater than that of nickel was further indicated in experiments with higher concentrations. Exposure to a solution of N/500 nickel acetate was followed by a return of contractions when the intestinal segments were transferred to pure Locke's solution. Sometimes rhythmic contractions reappeared while the intestine was still in contact with the salt. That the depressing effect was more marked with solutions of zinc malate than with nickel was also shown.

when weak contractions only, much reduced in frequency, were observed as the ileum was transferred from N/2,000 zinc malate in which it remained 20 minutes, to pure Locke's solution. In another experiment however the jejunum made almost a complete recovery in pure Locke's solution in which it remained 8 minutes. The difference in the reaction of the intestine of the cat to zinc and nickel was not so clearly defined as in the case of the rabbit but the tendency to greater depression as a result of treatment with zinc was shown as recovery in Locke's solution occurred after being treated with N/2,000, N/1,000

Fig. 13. Rabbit 1924. 1 B. A, shows stimulating effect of pilocarpine after four minutes in N/500 nickel acetate. B, contractions in Locke solution after 33 minutes in nickel acetate.

and N/500 nickel acetate, whereas the same concentrations of zinc malate had rendered the cat's intestine incapable of recovery in pure Locke's solution. Previous observations on the reactions of the intestine of different animals to various substances were made by Kuyer and Wijsenbeek (22) who have likewise shown that the intestine of the cat and rabbit differ in their behavior towards tyramin as this substance depressed the former and stimulated the latter. According to Magnus (23) and Kress (24), marked differences were also observed in the effects of physostigmin and nicotine on the isolated intestine of various animals.

That zinc is more toxic than nickel was also shown very distinctly by the reaction to pilocarpine and barium chloride. Thus the effect of pilocarpine was slight after 25 minutes exposure to N/5,000 zinc malate and 15 minutes exposure to N/2,000. When subjected to the action of N/1,000 zinc malate for 15 minutes little stimulation with pilocarpine could be obtained. This condition was first observed in experiments with N/200 nickel acetate, but stimulation with pilocarpine was pronounced when concentrations of N/1,000 and N/500

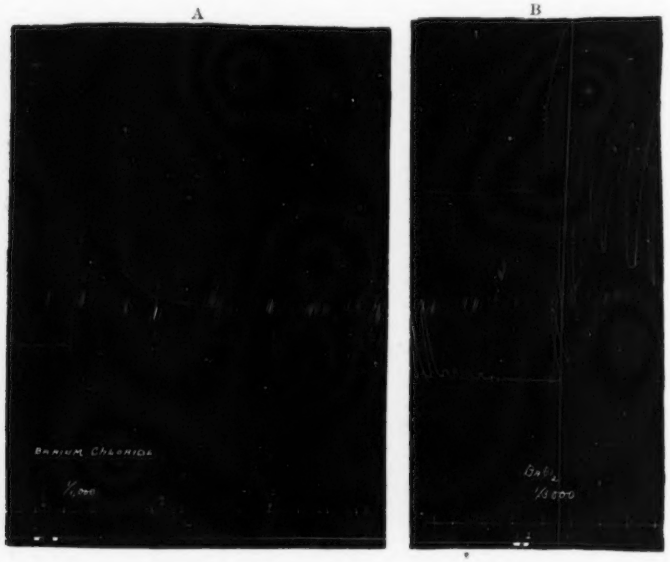

Fig. 14. Rabbit 1903. I. 1, jejunum. 2, ileum. A, reaction to barium chloride after 18 minutes in N/1000 zinc malate. B, 1926 reaction to barium chloride after about 18 minutes in N/200 acetate.

nickel acetate were employed. The reaction to barium was still present in intestinal segments treated with N/100 nickel acetate. The ileum exhibited a fair degree of rhythmic contractility when treated with 1/500 $BaCl_2$ after 25 minutes suspension in N/100 nickel acetate, the jejunum did not show rhythmic action but tonus rose as a result of $BaCl_2$ treatment. Barium chloride produced powerful contractions after 18 minutes exposure of the intestinal segments to N/200 nickel acetate. The effect of $BaCl_2$ was much less when they had been acted

upon by N/1,000 zinc malate for 18 minutes (1903 I and 1926), thus furnishing additional evidence of a difference in the behavior of these two metals which suggest a different mechanism of their toxicity. This is also indicated by the recovery and stimulation which often occurred in intestinal segments suspended in dilute solutions of nickel acetate but which was seldom seen in experiments with zinc malate. It may be recalled that Voegtlein (21) suggested on the basis of experiments on the isolated heart that the mode of action is the same for all heavy metals.

The reaction of different parts of the intestine to zinc and nickel was found to vary considerably in some of our experiments, the ileum and colon being less readily affected than the jejunum and duodenum. Contractility often disappeared in the latter while it persisted for a considerable time in the ileum and colon. Siccardi (25), who experimented with the isolated intestine, observed that lead acetate may produce a rise of tonus in segments of the small intestine and depression in the large intestine and rectum. In experiments with chenopodium, however, the writers (26) found that the ileum is less resistant than other parts of the small intestine. The recent work of Alvarez (27) on the contractility of various parts of the small intestine, indicating that frequency of rhythm diminishes with distance from the pylorus, would explain the results we obtained with chenopodium and those of Siccardi with lead. The action of zinc and nickel on different parts of the intestine probably depends upon different mechanisms.

SUMMARY AND CONCLUSIONS

1. Zinc is a powerful depressant, exposure to very dilute solutions for 30 to 45 minutes causing decreased contractility of the intestine from which it only partly recovers on changing to pure Locke's solution.

2. Medium concentrations of zinc may completely and permanently abolish contractions.

3. Dilute solutions of nickel acetate may stimulate intestinal contractility after causing primary depression. Only the more concentrated solutions inhibit muscular activity but considerable improvement was observed when changed to pure Locke's solution.

4. That nickel is much less toxic than zinc was also shown by the reaction of the intestine to barium chloride and pilocarpine, response to these agents being obtained after treatment with a much more concentrated solution of nickel acetate than of zinc malate.

5. The reaction to barium persisted longer than the response to pilocarpine after the intestine was exposed to the influence of zinc and a concentrated solution of nickel acetate.

6. The intestine of the cat is more resistant to the action of zinc than that of the rabbit. The difference is not so marked in experiments with nickel.

7. The ileum and colon were more resistant both to the action of zinc and the depressing effect of higher concentrations of nickel.

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NERVE CONDUCTION, AND OTHER REACTIONS IN CASSIOPEA

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Received for publication December 3, 1915

The rhizostomous scyphomedusa *Cassiopea xamachana* of Florida lives in shallow semi-stagnant lagoons wherein the temperature, salinity, and CO_2 are subject to considerable range. Thus it is one of the most favourable of marine animals for physiological studies, being so well adapted to aquarium conditions that the death rate is practically *nil* even though the experiments may continue for more than a month. Thus the medusa can be starved for at least 41 days in doubly filtered sea-water, its final weight becoming reduced to 1/27 the original. The gelatinous substance of the bell is consumed during starvation, and there is no differential consumption of body substances as in vertebrates, but one and only one set of substances are consumed. Thus the loss of weight each day is proportional to the body weight at the beginning of that day, and

$$y = W\,(1-a)^x$$

where y is the weight at the end of x days, W is the original weight when starving began and a is a constant being usually about 0.056 (1).

The medusa can survive a concentration of carbon dioxide in the sea-water which would be fatal to most other marine animals, and it may be kept for six weeks or more in the dark, at the end of which time its commensal plant cells will have largely disappeared and the medusa loses its olive green color and appears bluish and translucent, but it remains pulsating despite the fact that its plant cells are no longer able to reduce the CO_2 produced by its activity.

Small young medusæ pulsate more rapidly than large ones, and if a small and a large one be grafted together so that the nervous network of the subumbrellas comes into contact the two medusæ pulsate in unison, the small active individual initiating every pulsation in accord with Loeb's rule that in a physiological series the rate of activity is

that of the fastest member. Hence if we pinch the larger medusa it becomes the more rapid, and the controls the movements of the complex. Also as observed by Eimer (2) and Romanes (3) and as studied quantitatively by Cary (4) the marginal sense organs which initiate each pulsation are enabled to maintain a faster rate when enervating a large than when attached to a small area of tissue. Thus the rate of the two grafted medusæ is faster than that of either when cut apart. Also Cary (5) finds that the sense organs have still another function for they hasten the initial stages of regeneration in case of injury, and this action is independent of the rate of pulsation of the medusa. Even a single one of the normally 16 or more sense-organs can maintain the rate of pulsation and hasten the early stages of regeneration, and Tashiro (6) finds that the rate of metabolism measured by CO_2 production is decidedly more rapid in medusæ with even a single sense-organ than in those pulsating without sense-organs. Evidently these marginal sense-organs exert a control over the metabolic activities of the medusa. Each sense organ is a finger-shaped hernia-like diverticulum of the general gastro-vascular space of the medusa and contains an ectodermal ocellus, and an entodermal mass of crystalline concretions. Mayer (7) finds that these concretions are a uric oxalate of calcium and they augment in mass as the medusa grows older. He therefore, believes that it is the function of these sense-organs to maintain a slight local excess of sodium at the nerve centers of the medusa, and as sodium is well known, from the work of Ringer Loeb and others, to be a powerful stimulant its presence would suffice to produce the periodic response of pulsation.

The chemical equation in so far as the important elements, the calcium oxalate and sodium chloride are concerned, may be written as follows:

$$Na_2C_2O_4 + CaCl_2 = \quad 2\,NaCl + CaC_2O_4$$

It appears that a soluble uric oxalate of sodium is being constantly manufactured in the distal end of the sense-club, and this precipitates the calcium which enters the sense-club from the outside, thus setting free a local excess of sodium chloride at the nerve center. The probability of this being the case is seen when we cut off the distal end of the sense-club, thus paralyzing the subumbrella tissue, but pulsations are restored if we flood the stump of the sense-club with a solution of 2 grams of sodium oxalate in 1000 cc. of sea-water, or with sea-water containing a corresponding excess of NaCl. Moreover when the sense-

clubs are cut off they regenerate and pulsation commences when the calcium oxalate crystals begin to form. As the formation of uric acid, urates, and oxalates is well known in animals there is nothing remarkable in this process.

Of the 16 or more marginal sense-organs, one only, the one which for the moment works the fastest, controls the rate of pulsation. The medusa normally pulsates at a slow but fairly regular rate, but an extra pulsation or a temporary quickening of the rate is commonly followed by a compensatory pause. Indeed the medusa when in systole is relatively insensitive to stimuli, and despite the fact that the pulsation is neurogenic, the differences between the activities of the medusa and those of the vertebrate heart are almost wholly of degree rather than of kind. Bethe (8) in a remarkable series of experiments makes this quite clear.

As is well known to physiologists through the work of J. Loeb and others, a slight excess of sodium, potassium, acid (H) or alkali (OH) causes the rate of pulsation to be augmented, but in greater concentration of these ions it is diminished. Thus a weak concentration of the hydrogen cation such as in carbon dioxide is a stimulant to the nerves, and causes muscular contraction, but in stronger concentration it becomes toxic and the muscles relax. Thus if there be rhythmically beating cilia overlying the muscles as in ctenophores, a slight excess of H or of OH causes muscular contraction, thus intensifying the skin-tension and pressing upon the cilia-bearing cells, and at once the cilia, being very sensitive to pressure, stop beating. Then if we increase the concentration of the acid or alkali, or prolong its action, the muscles become relaxed and the cilia relieved of pressure may pulsate at an abnormally rapid rate (9). The same effects are produced by an excess of sodium or potassium which in weak concentration stimulate nervous and muscular activity but in stronger solutions are depressant. A very different effect is produced by magnesium which is depressant in any concentration and the first effect of which is to relax the muscular tonus, thus relieving the skin-pressure upon the cilia and permitting them to beat with abnormal rapidity. The fact is that the cilia-bearing cells, although affected in the same way as are the nerves and muscles by H, OH, Na, Ca, K, or Mg, are not so sensitive and thus when freed from pressure may even appear to be stimulated, the depressant effect of the ion being more than offset by the relaxation of the skin-pressure.

As with the cilia so with the nerves and the muscles, their activities are affected in different degrees by the cations of the sea-water. Thus

magnesium depresses the activity of the muscles more than it affects the nerves,[1] but Harvey (10) finds that NaOH, KOH, $Sr(OH)_2$ and certain alkalies such as ethylamine and tetraethylammonium hydroxide depress the nerves more than the muscles, so that finally only slow myogenic contractions can be sent through the tissue in response to each stimulus. This however is exceptional for to most reagents the nerves are more resistant than the muscles so that if one cuts a strip of subumbrella tissue with a sense-organ left on at one end and then places the middle of the strip in 0.4 molecular $MgCl_2$ or 0.6 molecular $MgSO_4$ while the two ends of the strip are in natural sea-water, it will soon be seen that the contraction which starts at the sense-organ will pass through to the other end of the strip without any movement appearing in the middle. The same effect is produced by heating the middle of the strip to about 36° or cooling it to 12°C., or placing it in sea-water charged with CO_2; and all these experiments show that muscular response is one thing while the nervous stimulus is another, and the muscles may or may not respond to the nerve stimulus dependent upon their condition.

The present writer (11) found indeed that rhythmical pulsation could be started and maintained even when there were no marginal sense-organs. If for example we cut off all the marginal sense-organs the medusa is at once paralyzed, and responds only by a single contraction to each stimulus lapsing immediately afterwards into a state of inactivity. This was observed many years ago by both Eimer and Romanes, but they did not succeed in causing medusæ to pulsate rhythmically and spontaneously after their marginal sense-organs had been cut off. This however can be done if we first remove the sense-organs and then cut any circuit-shaped strip of subumbrella tissue. If then a contraction wave be started by mechanical, chemical, or electrical stimuli so that it goes in *one* direction it cannot escape from the circuit of tissue and being entrapped must continually pass through it at a practically uniform rate, provided the chemical and physical conditions of the environment remain unchanged. Harvey (12) found that such a contraction wave may course for eleven days through the tissue with no appreciable decline in rate, traveling 457 miles during this time, the average rate of nerve conduction being 440 mm. per second at 28.9°C. Mayer found that such contraction waves in tissue isolated from the central nervous system could be produced in other medusæ such as *Aurellia*, *Dactylometra*, *Cyanea*, *Rhizostoma pulmo* or *Cotylorhiza*,

[1] The reverse is the case in mammals, as shown by Meltzer and Auer, and Joseph.

and also in ring-shaped strips cut from the ventricle of sharks, or the loggerhead turtle (13). Later Garrey (14) gave a good description of the same phenomenon in the ventricle of the loggerhead turtle evidently being unaware of the previous experiments made at Tortugas.

It is only necessary that the length of the circuit should be long enough to permit a sufficient interval of rest before the return of the entrapped wave. Even so a ring of subumbrella tissue pulsates, according to Cary (15), about three and one-half times as rapidly as does the normal medusa activated by its sense-organs, and Mayer found that it could respond by a contraction to each and every induction shock even when the shocks came at double this rate. We see therefore that the medusæ pulsate normally at only about one-seventh the rate they are capable of maintaining. As is well known tissue which has been in pulsation is thereby exhausted and must rest for a time before it can again respond to a constantly present stimulus. Thus rhythmical pulsation represents alternate fatigue and recovery.

When medusæ are pulsating normally with sense-organs intact a sudden shock or any very strong stimulus may cause the major part of the wave from the activating sense-organ to go to one side instead of spreading equally over the disk, and this starts a circuit wave which rushes around and around the disk at about two or three times the normal rate of the jellyfish. This shows that the mechanism designed to prevent the exhausting condition of an entrapped wave is imperfect. In fact the pulsation waves normally arise from one sense-organ, the fastest working one, and travel around the ring of subumbrella tissue in both directions "setting off" each successive sense-organ as the waves pass. Finally the two waves meet "front to front" on the opposite side of the medusa and being equal each to each annul one the other. If however one of these waves is more intense than the other it will overpower the weaker wave and then travel constantly around the circuit until interfered with by some other wave which advances against it, or until it is obstructed in the nerve net or the exhausted tissue can conduct it no longer. The muscles tire much more rapidly than the nerves and after an entrapped wave has gone for two or three days the muscular movement is barely discernible, but the nerves may conduct for a week or more maintaining a practically constant rate.

In the vertebrate heart the mechanism to prevent entrapped waves is much more perfect for here, as is well known, each pulsation arises in the region of the sinus venosus, then spreads over the auricles and finally into the ventricle. In the loggerhead turtle it travels in the compact external muscular layer of the heart, the cavernated interior

being passively squeezed as if it were a sponge. In fact the cavernated interior can be cut away and the peripheral muscular layer can still maintain a circuit wave if cut into the form of a ring and then activated by a single induction shock. No circuit wave can arise in nature for the pulsation normally dies out at the apex of the ventricle and cannot return over the old path, for the tissue must rest before it can again respond.

It is fortunate for physiological studies that we can maintain these entrapped circuit waves in tissues, for this enables us to study a *single* stimulus which in the case of the medusa maintains itself without apparent diminution for days. The stimulus is neurogenic in the medusa and is thus not easily exhausted, but in the vertebrate heart it is myogenic and in this case fatigue soon develops and the circuit waves soon die out, although Garrey (16) succeeded in maintaining such a wave in the ventricle of the loggerhead turtle for seven hours.

As is well known from the studies of the brothers Hertwig (17), and of Hesse (18), the nerves of scyphomedusæ form a network immediately under the ectodermal epithelium of the subumbrella and overlying the muscles. The nerve cells are usually bipolar and the fibers are non-medullated. In *Cassiopea* the subumbrella alone responds to stimuli, the exumbrella being so poorly supplied with nerves that its depressed centre may be flooded with corrosive sublimate without producing the least effect upon the regular rhythm of the medusa, provided the poison does not diffuse into the surrounding sea-water and thus reach the sense-organs and the subumbrella. Of all parts of the medusa the marginal sense-organs or rhopalia are most sensitive to stimuli, but even a ring which lacks sense-organs and is pulsating by means of an entrapped wave will respond by suddenly augmented muscular contractions when locally stimulated in any manner.

We must keep clear in our minds that under normal conditions the marginal sense-organs or rhopalia initiate neurogenic stimuli, and that this nervous stimulus secondarily affects the muscles causing them to respond, but the nervous stimulus can be made to pass through the subumbrella without causing the least visible response from the muscles, and conversely as found by Harvey the muscles may under certain conditions transmit a slow myogenic contraction even when the nerves have ceased to function.

The muscles are much more affected by temperature, salinity, or excess or deficiency of sodium, potassium, calcium and magnesium than are the nerves. For example if the medusa be heated from 29° to 38°C.

the muscular movement is barely perceptible but the nerves now conduct at about one and one-half times their former rate. Similarly if the sea-water be diluted by mixing 33.3 parts of sea-water with 66.6 parts by volume of distilled water the nerves still conduct at about 29 per cent the normal rate but the muscular movement is so reduced that it is usually impossible to record it on a kymograph. Also the muscular movement is at once brought to a stand-still by placing the medusa in 0.4 molecular magnesium chloride or 0.6 molecular magnesium sulphate but the nerves still conduct but with diminishing velocity. If the sea-water be diluted with 0.4 molecular magnesium chloride the decline in rate of nerve conduction is nearly the same as if we diluted the sea-water with distilled water as shown in the following table. This shows the remarkable insensibility of the medusa to changes in osmotic pressure.

TABLE I

COMPOSITION OF THE SOLUTION	A AVERAGE RATE OF NERVE CONDUCTION IN SEA-WATER DILUTED* WITH DISTILLED WATER	B AVERAGE RATE OF NERVE CONDUCTION IN SEA-WATER DILUTED WITH 0.4 MOLECULAR $MgCl_2$ DISSOLVED IN THE SAME DISTILLED WATER AS USED IN COLUMN A
Natural sea-water	100	100
95 cc. sea-water + 5 cc. distilled water	100.5	97.9
90 cc. sea-water + 10 cc. distilled water	95.89	95.3
80 cc. sea-water + 20 cc. distilled water	88.3	88.9
70 cc. sea-water + 30 cc. distilled water	81.4	78.1
60 cc. sea-water + 40 cc. distilled water	71.1	67.2
50 cc. sea-water + 50 cc. distilled water	56.31	55.4
33.3 cc. sea-water + 66.7 cc. distilled water	29.	

* This distilled water contained a slight amount of CO_2 and was thus acid and stimulating.

The hydrogen ion in weak concentration such as $1\ n \times 10^{-5}$ stimulates both muscular contraction and the rate of nerve conduction, the effect upon the muscles being more marked than upon the nerves, but in greater concentration acids soon become toxic and depress the activity of both muscles and nerves, especially the muscles; and it is interesting to see that Osterhout (19) presents a table showing that in plants a weak concentration of HCl is at first stimulating but later after a greater concentration of the H ion has passed through the plasma membrane of the plant it becomes depressant.

Amines and inorganic hydroides (20) are stimulating in weak concentration but as shown by Harvey (21) certain of them in stronger concentration become more depressant to nerve conduction than they do to muscular response so the muscles can still transmit a myogenic contraction after the nerves cease to function. Also I find that if sea-water be diluted with alkaline distilled water containing 0.75n 10^{-5} OH ion concentration the muscles still contract vigorously even in 50 per cent sea-water and 50 per cent of this distilled water, whereas in neutral or slightly acid distilled water the muscular response would be very feeble in this solution.

An interesting selective effect is produced by calcium. If the normal medusa be placed in an artificial sea-water[2] solution which however lacks calcium it ceases to pulsate for one to six minutes and the pulsations thereafter are only occasional and are separated by longer and longer intervals of time so that after a few hours all pulsation ceases and the medusa remains relaxed and inert; but even after a day or two of quiescence it can be almost instantly activated by restoring the calcium. Howell as is well known observed the same phenomenon in the turtle's heart in 1901.

Nerve conduction is, however, affected but little by the absence of calcium in the surrounding sea-water, the calcium of the tissues being sufficient to maintain a nearly normal rate in an entrapped wave for several hours.

A more striking phenomenon is seen when the medusa is placed in a partial sea-water solution which lacks magnesium,[3] or in sea-water containing an excess of calcium. After a few hours the medusa goes into clonic tetanus (22), and the muscles finally tear themselves into shreds; but even after 24 hours recovery of normal tonus and pulsation is very rapid if we add sufficient magnesium; for the Mg cation being depressant in all concentrations relaxes the muscular tonus (23). Calcium tetanus is purely muscular and local for if part of a strip of tissue be dipped beneath the surface of sea-water containing an excess of calcium it develops a local tetanus which does not spread to those parts of the

[2] 81.1 volumes 0.6 molecular NaCl
14.36 volumes 0.4 molecular $MgCl_2$
2.84 volumes 0.39 molecular $CaCl_2$
1.7 volumes 0.6 molecular KCl
Total 100.00

[3] A solution which resembles sea-water but lacks $MgCl_2$ would be made up by omitting $MgCl_2$ in the above solution.

strip which remain in normal sea-water. Moreover this tetanus cannot take place unless sodium as well as calcium be present for if we place a medusa in a pure calcium solution or in one containing the proportions of calcium, potassium and magnesium found in sea-water no tetanus develops. Thus there is reason to support the contention that sodium and calcium combine or become somehow intimately associated with each other in these reactions. In 1906 the present writer (24) observed that in medusæ calcium assists the sodium to overcome the inhibiting effects of magnesium, and this was discovered to be true by Blake and by Meltzer and Auer for mammals, and by Mines (25) for fishes. J. Loeb was the first definitely to determine that calcium ions can produce tetanus in muscle, but he went further and found in 1902 that the salts of monivalent cations exert toxic effects, but that these effects can be counteracted by slight amounts of salts with bivalent cations. Thus a weak concentration of the calcium ion can annihilate the toxic effect of a strong concentration of the sodium ion (26). Osterhout (27) shows also that a similar antagonism between the Na and Ca ions exists in plants, and that salts are also antagonized by acids; a condition first observed in animals by Loeb. So this relation is apparently general for all organisms.

Recently Loeb (28) finds that if the chlorides of sodium, potassium, calcium and magnesium be mixed in proportions found in sea-water and if experiments be made with solutions ranging from $\frac{1}{8}$ m. to $\frac{1}{2}$ m. the mixtures which maintain a normal activity in *Balanus* larvæ are those in which the concentration of the NaCl + KCl is about 35 times as great as the $CaCl_2 + MgCl_2$. "In other words the concentration of CaCl + MgCl required increases in direct proportion with the concentration of NaCl + KCl, thus following Weber's law."

But to return to the special consideration of *Cassiopea*, we see that its muscular activity is stimulated by the combination of sodium, calcium and potassium found in sea-water, but inhibited by the magnesium so that only a normal muscular tonus remains as a result. The muscles are therefore not stimulated into activity by the sea-water as a whole, but each pulsation arises as a result of a constantly present neural stimulus, probably sodium, in the marginal sense-organs. Thus the muscular tissue of the jellyfish is in a balanced medium containing stimulants, Na + K, and a depressant, Mg, which antagonize one the other. The Ca while itself a depressant becomes a sustainer of activity when combined or associated with the sodium.

For the nerves the conditions are quite different for we find that the decline in the rate of nerve conduction in diluted sea-water is nearly the same whether we dilute with distilled water, 0.9 molecular dextrose, or 0.4 molecular magnesium chloride. In other words the Mg ion is nearly as neutral as is distilled water in respect to nerves, but for muscular activity it is a decided depressant. The fact that both distilled water and 0.9 molecular dextrose produce each a similar effect upon the nerves when used to dilute the sea-water shows that osmotic pressure has little effect upon the nervous activity of the medusa (30).

It is also important to observe that magnesium takes practically no part in the control of the rate of nerve conduction, being nearly as inert as distilled water in this respect. The concentration of the sodium, calcium and potassium cations however determines the rate of nerve conduction. For example if we dilute the sea-water with distilled water which is nearly neutral but still slightly alkaline with an OH concentration between 10^{-6} and 10^{-7} the rate of nerve conduction in *Cassiopea* declines as follows: If y be the rate of nerve-conduction and x be the concentration of the sodium, calcium, and potassium cations in the surrounding sea-water.

$$y = 2.512\, x^{0.8} \qquad \text{and} \frac{x^{0.8}}{y} = 0.398$$

$$\text{or } \log y = \log 2.512 + 0.8 \log x.$$

This is identical in form with Freundlich's formula for *adsorption*, and suggests that the sodium, calcium and potassium cations are adsorbed and that these adsorbed cations conduct the nerve stimulus, the rate of which is by Wilhelmy's law proportional to the concentration of the cations which conduct it (31). As we have before observed if OH or H ions be present they will accelerate the rate of nerve conduction, but the significant fact appears to be that Na, Ca, and K are alone *sufficient* to conduct the nerve stimulus. In nature however there are probably always some free OH ions in sea-water and these would accelerate the rate. For example if we dilute the sea-water with alkaline distilled water having an OH concentration of $0.75\,\text{m} \times 10^{-5}$ the decline in rate is as follows:

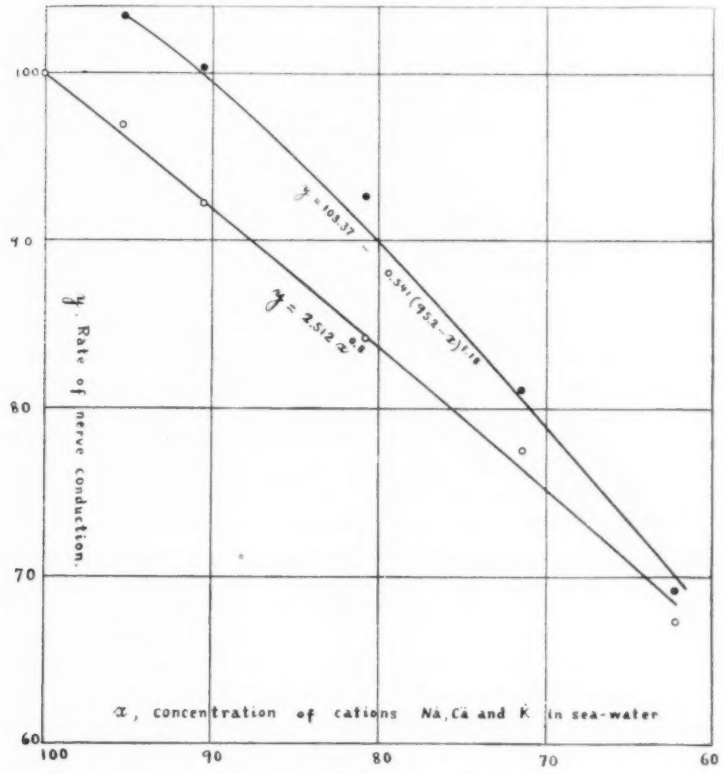

Fig. 1

We see that the OH anion in weak concentration, such as $6\ m \times 10^{-5}$, is highly stimulating to the rate of nerve conduction but that it becomes depressant in concentration higher than this. Moreover when OH is present in this concentration the formula for the rate of nerve conduction cannot be stated by the simple expression $y = a\,x^{\frac{1}{n}}$, but it assumes the form $y = B - d\ (C - x)^p$ where B is the highest rate of nerve conduction observed, C is the relative concentration of the cations Na, Ca and K corresponding to this highest rate and p is an integral exponent. When the distilled water is nearly neutral, however, as in Table II the equation for the rate of nerve conduction can be written

TABLE II

Rates of nerve conduction in Cassiopea in sea-water diluted with slightly alkaline distilled water

COMPOSITION OF THE SOLUTION	x RELATIVE CONCENTRATION OF THE Na, Ca, AND K CATIONS IN THE SEA-WATER	y OBSERVED RATES OF NERVE CONDUCTION. (OBSERVED y)	RATE CALCULATED FROM THE FORMULA $y = 2.512x^{0.8}$	THE RATIO $\frac{x^{0.8}}{\text{OBSERVED } y}$	RATE CALCULATED FROM THE FORMULA $y = 100 - 0.778\ (100 - x^{1.08})$
Natural sea-water	100	100	100	0.398	100
95 cc. sea-water + 5 cc. of nearly neutral distilled water	95.2	96.86 ± 0.66*	96.15	0.395	96.15
90 cc. sea-water + 10 cc. of nearly neutral distilled water	90.5	92.25 ± 1.62	92.32	0.397	92.29
80 cc. sea-water + 20 cc. of nearly neutral distilled water	80.8	84.2 ± 2.15	84.27	0.398	84.21
70 cc. sea-water + 30 cc. of nearly neutral distilled water	71.4	77.6 ± 1.82	76.38	0.397	76.29
60 cc. sea-water + 40 cc. of nearly neutral distilled water	62.2	67.5 ± 2.14	68.40	0.403	68.48

* Probable errors are stated as ± following a determination.

TABLE III

COMPOSITION OF THE SOLUTION	x RELATIVE CONCENTRATION OF THE Na, Ca AND K CATIONS OF THE SEA-WATER	y OBSERVED RATE OF NERVE CONDUCTION	RATE CALCULATED FROM THE FORMULA: $y = 103.37 - 0.541\ (95.2 - x^{1.43})$
Natural sea-water	100	100	
95 cc. sea-water + 5 cc. of alkaline distilled water	95.2	103.37	103.37
90 cc. sea-water + 10 cc. of alkaline distilled water	90.5	100.33	100.00
80 cc. sea-water + 20 cc. of alkaline distilled water	80.8	92 67	90.78
70 cc. sea-water + 30 cc. of alkaline distilled water	71.4	81.09	80.59
60 cc. sea-water + 40 cc. of alkaline distilled water	62.2	69.33	69.87

in either form and $y = a\, x^{\frac{1}{n}} = B - d\ (C - x)^{p}$ or in the case of Table II

$$y = 2.512\ x^{0.8} = 100 - 0.778\ (100 - x)^{1.019}$$

Thus the H or OH ions when present are not adsorbed but act as independent stimulating ions.

We venture to suggest that adsorption may play a fundamental rôle in nerve conduction and that the sodium, calcium, and potassium cations are attracted to the surfaces of negatively charged colloidal particles, for the sea-water and fluids surrounding the nerves being alkaline the colloidal elements of the nerve may be expected to carry a negative charge (32).

The number of the cations which the colloidal particles can capture and adsorb must depend upon the magnitude of the negative charges on the particles, and also upon the concentration of the cations in the surrounding fluid which in this case is sea-water.

A series of diagrams may serve to illustrate this hypothesis. Thus in figure 2 the nerve is represented by a row of negatively charged colloidal particles, for the colloid being normally alkaline the charge may be assumed to be negative. Line No. 1 shows the nerve in its resting stage wherein the negative charge of each colloidal particle tends to be partially neutralized by the adsorbed cations of sodium, calcium and potassium shown by + + +. The number of cations which each colloidal particle can capture and temporarily de-ionize (33) depends upon the potential of its negative charge and also upon the concentration of the cations in the surrounding fluid. For the sake of illustration we have shown three such cations attracted to the surface of each particle, but in reality the number must be greater than this.

Line No. 2 shows the beginning of a nerve impulse wherein the adsorbed cations of particle (A) have combined with some anions to form an ion-proteid, thus losing their positive charges and unmasking the negative charge of the colloidal particle. As a result other cations from the surrounding fluid (sea-water) are at once attracted and captured by the particle.

In Line No. 3 the reaction has passed on to particle (B) and its negative charge is unmasked, and thus the negative charge passes through the nerve at the rate of nerve conduction until each particle has lost its original cations, and then recaptured others from the surrounding fluid as in Lines 2–6, Line 6 representing the resting nerve after the reaction has passed through it.

Since 1899 Loeb (34) has maintained that physiological reactions are chemical phenomena associated with the formation of ion-proteids, but I think that while this is true for nerve conduction, it is only half the truth and that it is possibly a phenomenon of *adsorption* combined with that of an ordinary chemical reaction.

Loeb indeed is not antagonistic to the view that complex changes other than those of a simple chemical reaction may accompany nerve conduction, for he says: "We have to remember that all life phenomena are due to motions or changes occurring in colloidal substances" (35). No one however had reason to support the view that adsorption plays a part in nerve-conduction until the discovery of the change in rate of nerve conduction in *Cassiopea* in successive dilutions of sea-water suggested this to me as a possibility.

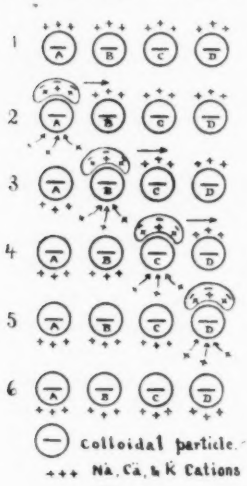

Fig. 2

My results lend no support to the theory of Sutherland (36) that the velocity of propagation of nerve impulse is that of a shear in the substance of the nerve. If this were the case its rate would vary with the viscosity of the surrounding fluid, but the decline in rate is practically the same whether the sea-water be diluted with distilled water, 0.9 molecular dextrose, or 0.4 molecular magnesium chloride.

Matthews (37), 1902, states that protoplasm consists essentially of a colloidal solution, and stimulation is accompanied by the passing of this solution to or toward a gel; and with these statements I am in accord. Matthews, however, believed the anions to be the stimulating ions, and he also thought the colloidal particles carry a positive charge. Later studies by many students have made it apparent that the cations are the more active agents in most physiological reactions, and that living protoplasm is normally alkaline and thus its colloids probably carry negative charges. Moreover the phenomena of adsorption were not well understood in 1902 and Matthews makes no mention of it in respect to nerve conduction.

It will be recalled that Harvey (38) showed that in *Cassiopea* the rate of nerve conduction augments in a right-line ratio as one heats the medusa from about 17° to about 35°C. beyond which it suddenly declines, and this result has been confirmed by Mayer (39). Also Knowl-

ton and Starling (40) found a right-line ratio for the excised hearts of dogs and cats when heated from 24° to 40°, the increase in rate being arithmetically proportional to the increment of temperature. Now if these were simple chemical reactions the rate should augment with rise in temperature in accord with the Van't Hoff law,

$$y = b\,(C)^z$$

where C is a constant usually from 2 to 3, and Z represents intervals of 10°C. Thus if at $n°$, $Z = 1$; at $(n + 10)°$, $Z = 2$; and at $(n + 50)°$, $Z = 5$, etc.

This law, which is based upon the previous work of van't Hoff for the speed of chemical reactions at ascending temperatures, was studied in detail by Snyder (41) for about 20 physiological activities and found to hold. It is therefore remarkable that it does not hold for the neurogenic pulsation wave of the medusa and for the myogenic activity of the vertebrate heart, for in both these reactions the rate augments more slowly than would be expected from Snyder's studies. If, however, the Na, Ca, and K cations which are essential to the reaction are adsorbed we would expect the rate to be reduced, for as Bayliss (42) points out heat dissociates an adsorption compound in a linear ratio with rise in temperature. Thus less is adsorbed at a high than at a low temperature.

In fact surface tension which is so intimately associated with capacity for adsorption also has a negative temperature coefficient, and it seems possible that some such factor may account for the observation of Weizäcker (43) that heat production in muscles is higher the lower the temperature, and muscular tension also has a negative temperature coefficient.

According to Bayliss the amount of substance adsorbed declines in a linear ratio of about 4.5 per cent for each 10° rise in temperature. At 17.5°C. the average rate of nerve conduction in *Cassiopea* is 54.5, and at 27.5° it is 103, instead of 152.5 as it would be did it follow Van't Hoff's law $y = 54.5\ (2.8)^z$, and it is reasonable to suppose that if nerve conduction depended solely upon the OH ion as the catalyzer of a chemical reaction its rate would be not far from 152.5. If however it depended solely upon the adsorbed cations Na, Ca, and K its rate might be expected to be $54.5 - 0.045 \times 54.5 = 52.05$ and the resultant rate would be $\frac{152.5 + 52.05}{2} = 102$ which is close to the observed 103.

At temperatures higher than 33° heat depression (44) becomes a

factor and must seriously depress the rate of all metabolic activities Thus the rate of nerve-conduction must soon reach a maximum and then decline rapidly as observed by Harvey. Thus the facts of temperature-reaction appear to accord with the hypothesis that the sodium, calcium and potassium cations are adsorbed by colloidal elements of the nerve.

It will be recalled that Lewis (45) found that inorganic salts lower surface tension at a water-hydrocarbon interface, hence such an adsorption as the one I postulate is possible; moreover the exponent 0.8 in the equation $y = 2.512x^{0.8}$ is not that of a partition coefficient for in this case if the exponent were stated as a vulgar fraction the denominator would be a simple integer. The exponent 0.8 is high for adsorption phenomena, but Mecklenburg (46) cites 16 cases of adsorption in which the exponent ranges from 0.167 to 0.965, being usually between 0.2 and 0.6. Hence 0.8 is not beyond the range of known cases of adsorption.

The question arises whether physiological activities other than nerve conduction may not be determined or influenced by adsorption. Goldfarb (47) has studied this question in relation to regeneration and finds that if we dilute the sea-water with distilled water which is acid with H_2CO_3 the rate of regeneration bears a general resemblance to that for nerve conduction when the sea-water is diluted with slightly acid distilled water. Later Goldfarb (48) found the same general curve for dilution with alkaline sea-water, but until his experiments have been completed and he has diluted the sea-water with neutral distilled water he prefers not to draw conclusions. Goldfarb's results support those of Loeb (49) that regeneration is somewhat more rapid in slightly diluted sea-water than in natural sea-water, but we do not know whether Loeb's distilled water was acid, neutral, or alkaline. *Cassiopea* is an exceptionally favourable animal for studies upon regeneration and Stockard (50) found that it conforms to Morgan's law that the deeper the level of an injury the more rapid the regeneration, for cuts made near the centre of the disk regenerate more quickly than those near the periphery. Stockard also found (51) that the regenerating tissue could develop at the expense of the body substance of the animal as in cancer, and in this he is in accord with King (52), who found that starfishes regenerate at the same rate whether starved or fed. In fact *Cassiopea* is an *exceptional* animal in that it is relatively insensitive to CO_2, salinity, and temperature changes, and thus hardy in confinement, but in all known physiological reactions it differs only in degree not in kind from other marine animals.

SUMMARY

It seems probable that sodium, calcium, and potassium cations are attracted by adsorption to the surfaces of negatively charged colloidal particles of the nerve.

When a stimulus passes these adsorbed cations combine chemically with proteid elements, thus neutralizing their electrical charges and revealing the negative charges of the colloidal particles.

Thus the negative charge passes through the nerve at the rate of nerve conduction; but it is quickly neutralized, for other sodium, calcium, and potassium cations are at once attracted to the surfaces of the colloidal particles, and thus in its resting stage the nerve is nearly neutral.

OH and H ions are not adsorbed, but if present in weak concentration they accelerate the rate of nerve conduction. In greater concentration they become depressants, the H being more toxic than OH in this respect. The presence of OH or H is not necessary to nerve conduction, and when free H and OH ions are absent the rate of nerve conduction is proportional to the concentration of the adsorbed sodium, calcium, and potassium cations.

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THE EFFECTS OF TESTICULAR TRANSPLANTS UPON VASOMOTOR IRRITABILITY

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Received for publication December 15, 1915

In a previous paper (1) it was pointed out that changes in functional as well as morphological processes follow the removal of the primary reproductive glands in male animals. It was further shown that blood pressure reactions to nicotin were constantly lowered as a result of gonadectomy; hence, the effects of castration were upon the sympathetic nervous system proper. At that time preliminary studies indicated that the presence of testicular grafts in such animals resulted in renewed activity of the vasomotor mechanism. This paper presents further data and conclusions on this point.

In the present, as in the former work, vasomotor reaction was taken as the criterion of the activity of the sympathetic nervous system, nicotin being used as a stimulant because of its selective action upon the ganglia (2) and vasomotor center proper (3). The pressure determinations were obtained from the femoral artery. Standard doses of nicotin, 1 cc., 1:2,000 solution, were flushed into the femoral vein with 0.9 per cent saline solution. The wounds were closed with continuous sutures and dressed with collodion cocoons. Ether anesthesia, administered by the open cone method, was used unaccompanied by other narcotics. The implants, which consisted of thin slices of partially decapsulated testicular tissue, were placed in a pocket formed among the deep dorsal muscles of the neck near the shoulder. The neck incision was found advantageous because of the ease of obtaining a deep muscular pocket for the graft, and of maintaining bandage dressings. The nature of the experiments necessitated a series of three operations upon each dog as follows:

1. The blood pressure and vasomotor reactions to nicotin were obtained on the normal dog, which was then completely castrated.

2. From 6 to 8 weeks later a second determination was made and a testicular graft placed at that time or shortly afterwards.

3. These implants were allowed to remain from 7 to 22 days, at the end of which time a third series of observations was made.

The following results and conclusions are based upon 87 blood pressure and vasomotor reactions to nicotin obtained from 20 anesthetized dogs. The numerical determinations are given in Table 1, and the average results shown graphically in figure 1.

TABLE I

Showing blood pressure, pressor effects of constant doses of nicotin and body weight determinations from three series of animals, before and after castration and after the reception of a testicular transplant. In series A are given the actual numerical readings of 9 dogs. Figures shown for Series B and C are averages computed from 33 readings made upon 11 dogs of a former work. Average results and per cent variations of Series A, B and C are based upon 87 determinations from 20 anesthetized dogs. All pressure readings are expressed in millimeters of mercury, body weight in kilos.

SERIES	A									B	C	AVERAGE	VARIATION
DOG NUMBER	1	5	6	7	8	9	11	14	15	6 dogs	5 dogs		
Nicotin 1 cc. of 1:2,000 solution.													*per cent*
Normal	90	53	57	58	60	44	46	×	×	26	44	53.1	
Castrated	27	20	26	22	22	64	10	26	32	17	25	26.5	−50.1
Grafted	86	42	31	×	28	44	17	45	41	×	35	41.1	+55.1
Blood Pressure Mm. of Hg.													
Normal	146	176	146	168	132	154	129	×	×	118	116	142.8	
Castrated	138	153	110	168	139	151	145	152	98	111	114	134.5	− 5.8
Grafted	146	174	110	×	138	155	148	150	98	×	114	137.0	+ 1.8
Weight in Kilos													
Normal	9.55	8.64	15.90	9.55	10.12	6.82	5.00	7.84	7.27	×	×	8.95	
Castrated	11.82	9.55	10.46	12.10	11.68	7.82	6.25	7.96	7.07	×	×	9.41	+ 5.7
Grafted	11.93	10.01	11.36	×	11.82	8.41	6.59	7.96	7.28	×	×	9.42	

As has been pointed out responses of the vascular system to injections of nicotin give evidence that one of the effects of castration is the lowering of irritability of the sympathetic nervous system. The entire series of 20 dogs, after castration, gave vasomotor responses to nicotin which averaged 50 per cent lower than reactions to the same drug before the removal of the gonads. That is, the normal responses to nicotin of 53 mm. of Hg. dropped to 26 mm. after gonadectomy. If this loss of irritability is brought about by the absence of the testicles, reinstatement of the missing parts should, at least, partially relieve the depres-

sion. Ten days after reception of the graft the vasomotor readings to nicotin, which at the end of 6 to 8 weeks after castration averaged 26 mm., rose to 41 mm., an increase of 55 per cent or a return to 77 per cent of the normal. According to these figures there remains a depression of 23 per cent below normal notwithstanding the presence

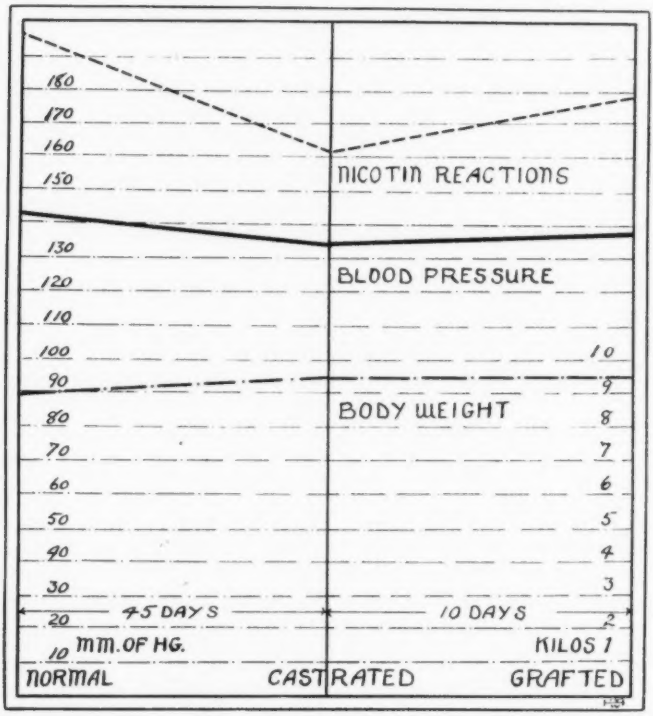

Fig. I. Composite curves of Series A, B and C showing the average blood pressure, vaso-motor responses to nicotin, and body weight of normal, castrated, and grafted dogs. Blood pressure readings are expressed in millimeters of mercury, body weight in kilos.

of the implanted materials. This condition may be due to a slow absorption of, or a deficiency of, the secretions because of the relatively small amount of testicular tissue present.

The tracings of figures No. II and III clearly show the effects of castration and the subsequent transolantation of testicular material

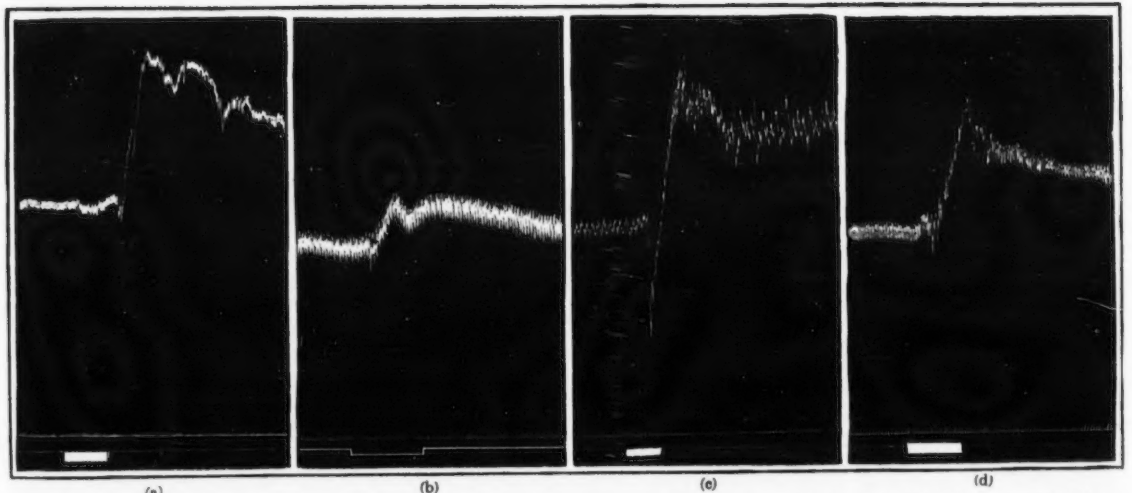

Fig. II. Reactions of Dog No. 1, Series A, to 1 cc. of 1:2,000 nicotin solution. a, March 2, 1915, before castration; b, April 17, 1915, after the operation; c, April 29, and d, May 6, 1915, after the reception of a testicular graft. The transplant was made immediately after having obtained reading b. Time, one second.

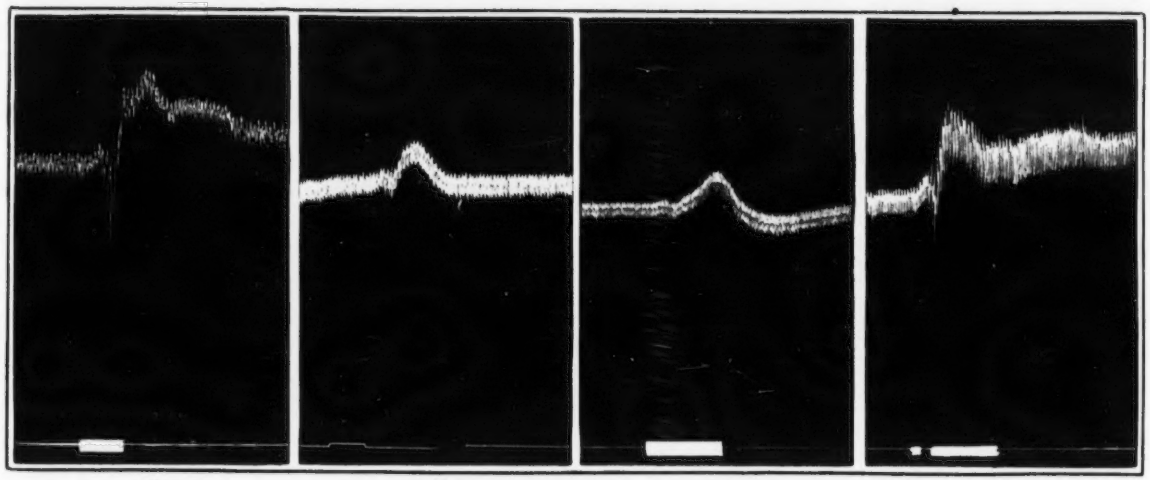

Fig. III. Reactions of Dog No. 5, Series A, to 1 cc. of 1:2,000 nicotin solution. a, March 3, 1915, before gonadectomy; b, April 20, and c, May 1, 1915, after the operation, and d, May 23, 1915, 22 days after the reception of a testicular transplant placed May 1, 1915. Time, one second.

upon the blood pressure and vasomotor reactions to nicotin. Tracing (a) of figure II, taken March 2, 1915, shows an initial blood pressure of 146 mm. and a pressor response to nicotin of 90 mm. Forty-six days after castration-tracing (b)—the blood pressure had fallen to 138 mm., 8 mm. below normal, and gave a nicotin reading of only 27 mm. On April 29, twelve days after the reception of a graft, the blood pressure, as shown in tracing (c), had returned practically to normal and gave a nicotin reading of 86 mm. Tracing (d), taken May 6, shows the continued effects of the implanted tissue. In figure III are shown four tracings from dog No. 5. Graph (a) is the reading from the normal dog. Tracings (b) and (c), both post-castration readings, show the continued lowered reactions to nicotin. Graph (d) shows the tracing obtained 22 days after the reception of a testicular transplant. The return of irritability in this case is apparent. These two figures show that the lowered reactions obtained by castration can be and are raised by the implantation of the secretory parts of the testis. So far neither the physiological life nor the period over which a graft can influence the nervous system has been determined, but our results show that they remain active for at least 22 days.

The slight fall in blood pressure following castration is easily within experimental error, however, this finding is constant and may be considered a result of the lowered activity of the vasomotor mechanism. The average normal blood pressure of 142.8 mm. of Hg. fell to 134.5 after gonadectomy, a lowering of 5.8 per cent, and was raised to an average of 137.0 mm. as a result of the presence of testicular transplants. Therefore, the presence of such implants in castrated dogs, while greatly increasing the vasomotor responses to nicotin, cause but little rise of the lowered blood pressure.

The present series of dogs showed an average increase of 5 per cent in body weight during the period of castration.

The present findings point to the conclusion that a direct relationship does exist between the internal secretions of the testis and the sympathetic nervous system. Castration results in a depressed activity of the nervous mechanism while subsequent establishment of the lost parts tends to lift the depression and at least partially to reinstate normal activity.

NOTE. The interesting observation was made during the work that normal dogs giving feeble nicotin reactions subsequently proved unusually susceptible to infections.

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STUDIES IN BLOOD PRESSURE ESTIMATIONS BY INDIRECT METHODS

I. The Mechanism of the Oscillatory Criteria[1]

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Received for publication December 17, 1915

INTRODUCTION

In 1901 and 1903 (1, 2, 3) the author reported the results of a series of experiments planned with the object of elucidating and evaluating some of the criteria employed in the estimation of the blood pressures in arteries by indirect methods. The introduction of new criteria for the indirect determination of the arterial pressures, the questions and the difficulties that have arisen in regard to the interpretation of the new as well as of the old criteria since then, have induced him to again turn his attention to this subject. During the past year experiments have been devised with a view not so much toward determining the accuracy of any particular criterion in the estimation of the blood pressures as toward the acquisition of more knowledge in regard to the principles underlying the criteria, to the relation of the criteria one to the other, and to the conditions modifying these relations.

We first turned our attention to certain of the moot questions bearing the pressure oscillations yielded by an artery to compression exerted from without. Although the relation of compression oscillations to the arterial pressures has been repeatedly investigated there still seems to be little unanimity of opinion in regard to a number of aspects of the subject. Consider, for instance, the views which have been and are still held in regard to the interpretation of the oscillatory criterion of the diastolic pressure. Thus upon the basis of carefully conducted experiments it is held by some (4, 5) that the

[1] Read before the Washington University Medical Society October 11, 1915. Some of the results were presented at the 66th Annual session of the American Medical Association, June 23, 1915.

diastolic pressure is indicated by the highest of the oscillations obtained, while others (6, 7), upon the basis of equally careful experiments, hold that highest oscillations are recorded under a compression that exactly equals the mean arterial pressure. Again, it is maintained that the last (compressing pressure falling) of the maximum oscillations and, when that is not sharply indicated, the first sudden diminution in amplitude (3, 8, 9) indicates the diastolic pressure.[2] It is scarcely conceivable that these differences, involving as they do comparatively simple relations and simple methods of experimentation, could be due entirely to erroneous observation. It seemed much more likely that under certain conditions all may be right, though no one has as yet attempted to reconcile the discrepancies.

EXPERIMENTS ON ARTERIES IN A CIRCULATION SCHEMA

Methods

We had had in mind for some time an attempt to determine where the difficulty lay when a simple method of undertaking the investigation became available through the description of a procedure suitable for the purpose by Brooks and Luckhardt (12). This procedure can

[2] MacWilliam and Melvin (9) regard the level of external pressure just after the abrupt diminution in amplitude has taken place as the correct guide to the diastolic pressure. There is, however, less difference between the criterion adopted by them and by the author than the former seem willing to admit. I have always in my readings of the diastolic pressure looked for the sudden diminution in amplitude. Thus I say: "The moment the pressure falls below the intravascular minimum, the amplitude of the pulsation, as a rule, diminishes abruptly. In such instances it is the *last* maximum series that is obtained at minimum intravascular pressure" (3, p. 68). Again: "As the pressure continues to fall the amplitude of the oscillations of the lever will continue to increase until the pressure on the arm falls below the intra-arterial minimum. At this moment the amplitude will diminish more or less abruptly. The pressure indicated by the manometer at this moment is equal to the minimum pressure" (3, p. 67). And again: " . . . the diastolic pressure corresponds with the abrupt diminution in amplitude" (10). The fact that the diastolic pressures given in my first publication on this subject are high is not to be attributed to the use of the criterion that MacWilliam and Melvin seem to suppose we used, but rather to the employment, at that stage of our work, of a narrow arm band. The values for the diastolic pressure which these authors quote from Howell (11) and which to their apparent surprise agree so closely with those they have obtained by their *new* criterion, were obtained in routine class experiments in which the broad arm band was used, under my direction. They have not been published elsewhere.

best be made clear by describing the essentials of the simple apparatus (fig. 1) we have put together for the purpose of applying it.

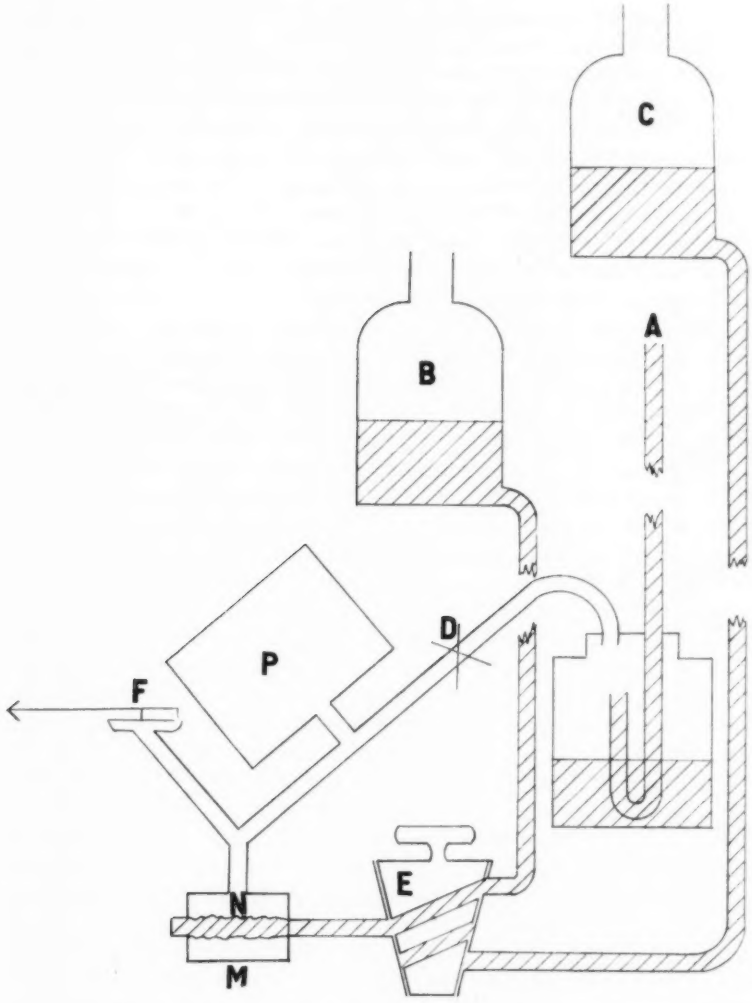

Fig. 1. Diagram of pulse schema. Description in text.

The "pulse" is produced by alternately putting the "artery," *N*, of the water-filled system into connection with one of two pressure bottles, one, *B*, determining diastolic, the other, *C*, systolic pressure. The "artery" consists either of rubbing tubing about 4 mm. in diameter made of *soft*, thin rubber dam, or of the fresh carotid artery of the dog. Both are stretched somewhat, the latter to its natural length, between the cannulae by which they are held in the axis of the glass compression tube, *M*. The compression tube, measuring about 6 cm. in length and 1.1 cm. in diameter, communicates with the pressure bottle above *A*, by means of which the pressure of the air in the compression chamber can be varied; with an additional air space, *P*, whose capacity can be readily varied so as to vary the compressibility of the compression chamber as a whole, and with a recording tambour, *F*, 1.5 cm. in diameter stretched with heavy rubber dam.

The steps in the procedure consist in first turning the stopcock, *E*, so as to throw into the artery one of the arterial pressures, either diastolic or systolic, then setting the extra-arterial pressure at the desired level by adjusting the height of bottle, *A*, and closing the stopcock at *D*. Then the stopcock, *E*, is turned so as to cause the other arterial pressure, either systolic or diastolic as the case may be, to exert itself upon the artery. At each external, or compressing, pressure records are made of the level assumed by the lever of the tambour, *F*, under each of the arterial pressures in the form of short horizontal lines placed one immediately above the other, one indicating the level of the crest of the oscillation, the other that of the trough. When this is done at compressing pressures ranging from below diastolic to above systolic, a series of couples of parallel lines is obtained from which curves can be constructed so as to show the pressure oscillations transmitted from the artery to the chamber under every grade of compression.

Discussion of the method. This method of investigating the principles underlying the oscillatory criteria has certain advantages over that used by MacWilliam and Melvin and by myself:

1. In the first place it eliminates any modifying influences that the time factors of the pulse may exert upon the oscillations. This difference is considerably to the advantage of the present method, for it is obvious that the possibilities of variation in the configuration of the arterial pulse render it difficult if not impossible to take into account all of the inertia effects due to them. The Brooks-Luckhardt method, however, by eliminating the time factor provides us with the pure effects of the fundamental factors of the compression oscillations; to these

it may be possible to add by inference such disturbing inertia and resistance effects as may result from differences in the duration of the several phases of the pulse.

2. Another difference consists in the absence of flow; the resistance in our schema is infinite. This again eliminates some of the difficulties in the interpretation of results which have confronted workers in this field (3, 9). To illustrate the significance of this factor we may consider first the conditions obtaining when there is no peripheral resistance. The compression chamber itself then determines the peripheral resistance. As the compressing pressure falls the central systolic pressure will first fall and then, when diastolic compression is reached, the central diastolic will also fall. Under conditions that obtain in actual practice, however, not alone does the peripheral resistance offered by the arterioles and veins increase relatively as more and more blood succeeds in forcing its way through the compression chamber, but in addition the absolute peripheral resistance itself is unknown. It is obvious that under such conditions an exact analysis of the results obtained is scarcely possible. Standard conditions can evidently be obtained only by making the resistance infinite as we have done in this series of observations. But not alone does peripheral resistance alter the central arterial pressures in the sense mentioned above—it also will influence the rate with which the blood flows into and out of the artery in the compression chamber: a high pressure (resistance) peripherally hastening the filling and delaying the emptying of that part of the artery. The Brooks-Luckhardt method, by eliminating the time factor eliminates most of the ambiguities attributable to these effects of peripheral resistance.

3. Another advantage possessed by this method consists in the exactness with which the arterial pressures, systolic and diastolic, can be measured: it is merely necessary to measure the height of the pressure bottles. The determination of the maximum pressure of a pulse is by no means a simple matter. A valved mercury manometer accomplishes this, we believe, only approximately; the least irregularity of the pulse, even though only occasional, causes the manometer to rest at a level that does not correspond with the general levels of the arterial pressures. Furthermore, it is not often while recording the maximum pressure that we can wait until the valved manometer ceases altogether to rise, although the error resulting from measuring the distance between the base line and the line at which the maximum manometer *tends* no longer to rise probably never is very great.

GENERAL STATEMENT OF RESULTS

The results we have obtained with this apparatus show that all of the divergent views that have been held with regard to the relation to the critical compression oscillations to the arterial pressures may under certain conditions be correct. Just at which extra-arterial pressure maximum oscillations will occur, or when, in relation to the arterial pressures, sudden changes in amplitude will appear depends mainly (1) upon the compressibility of the compression space, (2) upon the extensibility of the "artery," (3) upon the ratio of upper and lower conical closures to complete closure and, especially, when compressibility of the compression space is sufficiently small, (4) upon the phase of the pulse cycle in which the compressing pressure is brought to bear upon the artery.[3]

THEORETIC CONSIDERATIONS

The analysis of the theory of compression oscillations that led us to believe that these are the factors that determine the form of the oscillation record, and that furnished the incentive to this phase of our investigation may well precede the consideration of our results.

1. *Incompressible transmitting medium*

a. *Inextensible artery.* The simplest set of conditions may be considered first. The compression chamber is rigid and filled with incompressible fluid; the indicator of the compression oscillations is moved by a minimal translocation of fluid; the artery is inextensible; as it collapses there is no tendency for it to stretch between the supporting cannulae; nor does it present a conical closure to the pulse. Then, the circulatory conditions remaining constant, the compression oscillations due to the pulse will depend only upon the external pressure and the phase of the pulse cycle in which this pressure is applied to the artery. The results obtained under these ideal conditions may be

[3] MacWilliam and Melvin note (9, p. 165) that "the effects of external pressure upon the internal pressure are influenced to some extent according as to whether the air in the compression tube is, or is not in continuity with the air in the . . . . rubber bag used for raising the pressure . . . . When the reservoir of air in the compressor is shut off there are notable differences in effect according as to whether it is shut off during the systolic or the diastolic phase." They, however, fail to note whether there were any coincident effects upon the compression oscillations obtained from the artery.

made clear by referring them to a system of coordinates (fig. 2). If the abscissae are made to represent the compressions applied to the artery and the ordinates the variations in the compression pressure effected by the pulse, the line along which the pressure in the com-

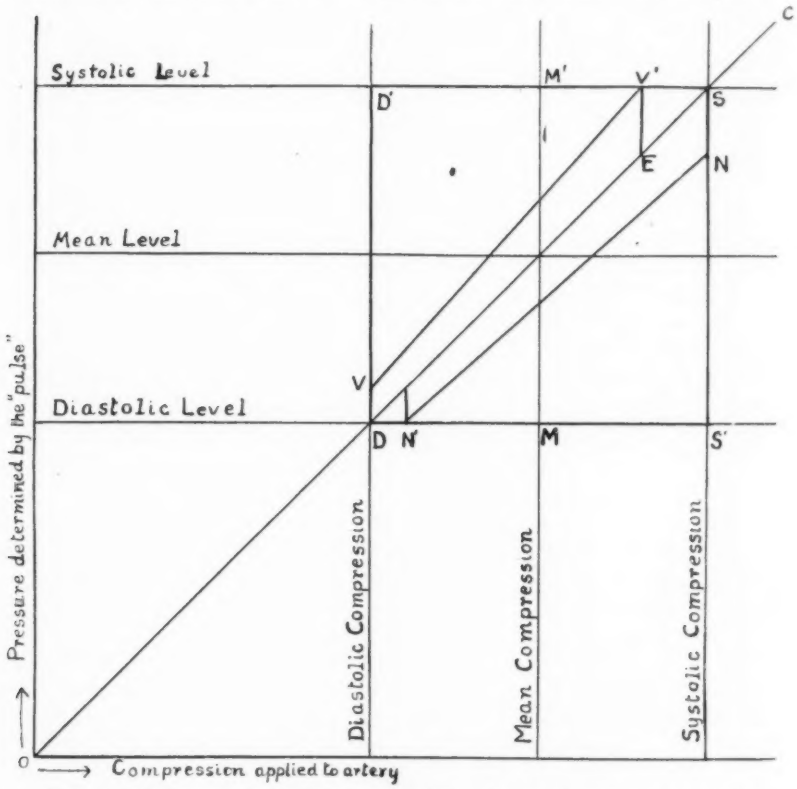

Fig. 2. Diagrammatic representation of the theory of compression oscillations.

pression chamber would rise, in the absence of pulsations, would be represented by *OC*. Now let the compressing pressure be applied in each observation while the pulse of the amplitude subtended by the "systolic-diastolic levels" is in its diastolic phase. Then during the rise of compressing pressure from *O* to the "diastolic level" the com-

pression oscillations will be nil, for during this period the artery remains full and its inextensible wall prevents any change in its volume with the pulse. At compressions exceeding the diastolic level the artery will be collapsed by the application of the pressure from without, but the pulse can now transmit its pressure through it to the compression chamber; therefore, from this level, and until the compressing pressure attains the systolic level, each pulse will raise the pressure in the compression space from its gradient, *DS*, to the systolic level, *D'S*. This will continue to occur until the compressing pressure, *OC*, exceeds the "systolic level," *D'S*, when there will no longer be in the compression space any pressure variations due to the pulse. Under these circumstances, therefore, the complete record of the compression oscillations will have the form of the triangle, *D D'S*.

If, on the other hand, the compressing pressure be applied each time during the systolic phase of the pulse the complete record of the oscillations obviously will have the form *D S'S*. It is thus seen that in one case maximum oscillations are obtained when the compressing pressure equals the diastolic pressure, in the other, when it equals the systolic pressure; and that (compressing pressure rising) in one case the diastolic pressure is indicated by an abrupt increase in the amplitude of the oscillations, in the other by the appearance of oscillations which then gradually increase in amplitude.

Again, if the compressing pressure be applied while the arterial pressure is at its mean level, the oscillation record would have the form of the figure *D M M'S*: maximum oscillations are recorded when the compressing pressure equals the mean arterial pressure.

b. *Extensible artery.* Making the artery elastic, the other conditions remaining as before, modifies the results by permitting the transmission to the compression chamber of some of the pulse pressure while the compressing pressure is below the diastolic level. And when, in addition, the attachment of the artery to the cannulae in the compression chamber results in its being stretched when compressed, the middle parts closing first, the lateral parts only after the application of considerably higher compressing pressures, not alone is this effect still further increased, but in addition pulsation is still transmitted to the compression chamber even when the compressing pressure exceeds the systolic pressure.

2. *Compressible transmitting medium*

Nevertheless, as long as the compressibility of the compression space is limited, extensibility of the artery plays a relatively unimportant part. But as the compressibilty of the compression space increases, the elasticity of the artery becomes a more and more important factor, since then the range of the pressure oscillations in the compression chamber is relatively slight and the arterial walls rather than the compressing pressure therefore support the pressure exerted from within the artery.

a. *Inextensible artery.* In discussing the influence on compression oscillations of a compression space of high compressibility it is, however, convenient to again consider first the case of a wholly inextensible artery. The air-filled compression space, we will first premise, is so large that reducing it by an amount equal to the volume of the artery increases its pressure by a relatively small fraction, say *D V* (fig. 2), of the arterial pulse pressure, *D D'*. It is also convenient to assign a relative value to the pulse pressure: let it, for the sake of simplicity, be equal to the diastolic pressure; and let the compressing pressure for the present be applied each time while the pulse is in its diastolic phase.

Upon consulting the diagram, it will be seen that with each pulse the artery under these conditions will open practically through its full diameter from the moment the compressing pressure exceeds the diastolic and until, as a compressing pressure indicated by *E*, the filling of the artery raises the pressure in the compression chamber to the systolic level, *V'*. Inasmuch as the volume of incompressible fluid entering the artery is practically the same throughout the diastolic-systolic range of compression and since at this time, as premised above, the compressing pressure is nearly twice that which obtained at *D*, the pressure in the compression chamber will be raised almost twice as high by the pulse at *E* as at *D*; for the rise of pressure determined by the addition of a given volume of incompressible material to a confined, gas-filled space is proportional to the pressure of the gas filling the space. But when the compressing pressure rises above *E*, the increase in the volume of the artery produced by the pulse is stopped by the equal counterbalancing compressing pressure that is developed, and the pressure oscillations then diminish in amplitude in the form of the figure *E V'S*. The configuration of the oscillation record is then *D V V'S D;* maximal oscillations are recorded close to, if not at, systolic compressing pressure.

The configuration of the compression oscillations obtained when, under the same conditions, the compressing pressure is applied during systole, is represented by *S N N' D S;* for then with each pulse the artery ranges between its full, systolic size and complete collapse, excepting where at low pressures the decrease in the size of the artery lowers the compressing pressure to the "diastolic level." Under these conditions, therefore, maximal oscillations are recorded exactly at systolic compression.

b. *Extensible artery.* If now the inextensible tube be replaced by an extensible tube and one whose extensibility is the same at all pressures within the range here employed, the "full" size of the artery will no longer always be the same but will vary with the difference between the compressing and the systolic pressures; that is to say, the artery will be larger at diastolic compression than at systolic compression. If this were the only factor, it is obvious that there would be a tendency, more decided when the compression is applied during the diastolic than the systolic phase of the pulse, for oscillations to be maximal when the compression equals the diastolic pressure. This effect would add itself to the effects depicted in the preceding paragraphs, produced by the filling of the tube up to its full, but undistended state. With a tube of sufficiently small undistended bore and of sufficient extensibility the oscillations at *D V* might well exceed in amplitude the oscillations at *E V'*. This tendency would be interfered with, though not necessarily counteracted, if the tube, like relaxed artery, were more distensible at low than at high internal pressures; for at low compressing pressures the distension attained would be then greater relatively than at high compressing pressures.

c. *Influence of the conical closures.* Finally, in order to match the conditions under which the artery oscillates in a compression chamber it is necessary to consider the influence the upper and lower conical ends of the compressed artery exert upon the oscillations. We will consider only the case of a large compression chamber. The deformation of the artery (and tissues) necessary to bring the walls of the artery together uses up some of the pressure exerted by the chamber (8). At the same time, owing to the attachment of the tube to the cannulae in the chamber, the chamber does not at its edges transmit to the artery the full pressure of its contents.[4] Consequently the

[4] Under natural conditions the resistance of the tissues and the way in which the arm bag transmits its pressure to the arm bring about a similar state of affairs.

ends of the compressed artery close gradually as indicated by the light lines in figure 3.

The length of the upper conical closure will vary somewhat with the arterial pressure and with the bore of the artery. Let us say that at a compression exceeding the systolic arterial pressure the apex of the cone stands at *a* (fig. 3) during diastole but descends to *b* during systole. It is obvious that as the compressing pressure diminishes both *a* and *b* will move down the artery and the distance between them will increase. Let us assume that at a compressing pressure just exceeding the arterial systolic pressure, the apex of the cone oscillates with the pulse between *a'* and *b'*. Now, when the compressing pressure falls just below the systolic level, practically the whole of the anacrotic limb of the pulse will be expended in pushing the apex of the cone from *a'* to *b'*; the very crest of the pulse will, however, act to open the rest of the compressed artery to its full but unstretched bore,

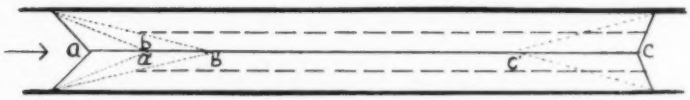

Fig. 3. Schematic representation of the movements of the walls of an artery under different compressing pressures. Heavy lines, systolic limits; light lines, compressed limits; broken lines, undistended bore; dotted lines, position of walls at upper and lower ends of compressed segment.

as shown in the figure by the broken lines, provided the time during which the arterial pressure exceeds the compressing pressure is sufficient to permit of the entrance of all of the blood needed to effect this change in volume. With further decrease of the compressing pressure the downward motion of the diastolic position of the apex of the cone continues, while the position reached by the cone when the artery opens remains fixed at *b'*, for this is the place where the artery closes at the moment the compressing pressure just exceeds the pressure in the artery, it matters not in what phase of the pulse cycle that balance of pressure develops.

The length of the lower conical closure depends upon the pressure in the artery below. It will begin to elongate upward when sufficient blood passes through the compressed artery to cause the pressure below to rise. When the artery beyond is not occluded, but possesses a peripheral resistance, this would become an important factor only toward the close of the systolic-diastolic period. The lower cone would practically cease to be a factor below diastolic compression.

Summary of the volume changes in an artery compressed in a large compression chamber, and their relation to compression oscillations

It has been deduced that pressure oscillations in a compression chamber containing a relatively large volume of air are dependent primarily upon the volume oscillations in the part of the artery compressed. Now the volume oscillations in that part of the artery are known if we know (a) the volume of the artery during diastole and (b) the increase in volume during systole. We will therefore consider the factors that have been mentioned above, together with certain additional ones, in relation to their effect (a) upon the diastolic or basal volume and (b) upon the systolic volume of the compressed artery. The factors are enumerated in tabular form (Table I) in the order in which they become effective while the pressure upon the artery is steadily decreasing.

It should be repeated here that the pressure changes in the compression chamber determined by these volume oscillations will be proportional to the pressure in the compression chamber. Therefore, throughout the entire range of falling compression there will be, in addition to all the factors mentioned in the table, a tendency for the pressure oscillations to steadily diminish in amplitude. If therefore, with falling compression, an increase in volume determined by the pulse is to cause an increase in the associated pressure oscillation, the volume change must be more than sufficient to offset the diminished effectiveness of the volume change on the pressure.

The volume tendencies given in the table have been fitted into a diagram (fig. 4, described in legend) which shows the volume oscillations thus theoretically derived. The diagram, it will be noted, presents a striking resemblance to the oscillation record usually obtained in blood pressure observations. But we hasten to add that, as respects steepness, the gradients of this diagram have been chosen arbitrarily, though in keeping with the theoretical considerations, for the very purpose of determining whether it would be possible to so derive such a resemblance. It is obvious, however, that the gradients might so vary with respect to each other as to produce very decided variations in the general configuration of the oscillation record. For instance, if the volume gradients, upper and lower, in the systolic-diastolic phase of decompression did not diverge sufficiently, the oscillations of the volume might actually produce pressure oscillations that decreased in amplitude. Such a diminution in divergence of the gradients might

TABLE I

Giving the factors determining volume changes in an artery in a compression chamber during decompression

COMPRESSING PRESSURE	DIASTOLIC VOLUME	SYSTOLIC VOLUME	VOLUME OF BLOOD MOVED BY PULSE (DIFFERENCE BETWEEN DIASTOLIC AND SYSTOLIC VOLUMES)
1. Falling to systolic level.	Diastolic position of apex of upper cone shifts from *a* to *a'*, gradually increasing the volume of blood.	Systolic position of apex of cone shifts from *b* to *b'*, increasing the systolic volume more rapidly than the diastolic volume.	Gradually increases.
2. At systolic level.	Same as at close of 1.	Increases momentarily† as far toward undistended bore of artery as time permits.	Suddenly increases.
3. From systolic to diastolic level.	a). Apex of upper cone continues to move toward *b'*, increasing volume of blood. b). Toward close of this period apex of lower cone begins to move upward, increasing volume of blood.*	a). Increases, but at a decreasing rate, through stretching of the arterial wall, and possibly through the increase in the time† the artery is open. b). As under a) above.	a). Increases rapidly at first, then more and more slowly (see below). b). There is a tendency, which may or may not be sufficient to overcome 3a) above, for the volume to decrease.
4. At diastolic level.	Marked increase as artery fills to undistended bore.	Same as at end of 3 b).	Suddenly diminishes.
5. Falling from diastolic level.	Increases steadily, but at decreasing rate, through gradual distension of artery.	Continues to increase as under 3, but the rate of increase, owing to higher pressure, is slower than diastolic increase.	Diminishes but at a decreasing rate.

* At this time also the elastic resistance of the artery to compression may begin to counterbalance a part of the compressing pressure, with the result that a certain amount of opening of the artery may occur during diastole where the walls happen to be sharply creased, thus causing a further increase in the volume of the blood.

† When the pulse has the configuration of the arterial pulse.

be due either to an increase in the steepness of the diastolic gradient, or to a decreased steepness of the systolic gradient. In a relatively wide, or short, artery, for instance, the steepness of the lower gradient might be increased through the increased importance assumed under such conditions by the upper conical closure. By increasing the length of the artery, however, the upper gradient might possibly be increased

to such an extent as to completely submerge this effect of the conical closure. Again, the upper gradient presumably would be less steep in the case of an inelastic artery. Indeed, we have shown in another place (3) that the oscillations obtained from an inextensible tube do not increase in amplitude nearly so rapidly in the systolic-diastolic range of compression as do those derived from an inextensible tube. And again, should the gradients in the earlier part of the systolic-diastolic phase of decompression be separating at such a rate as

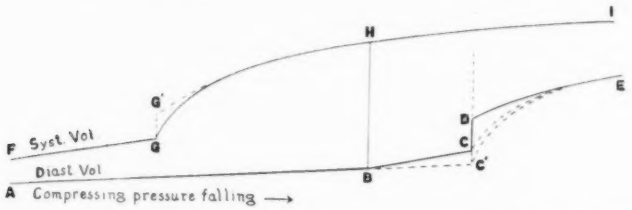

Fig. 4. Diagram illustrating the inferred volume changes of an artery in a compression chamber. The main factors concerned in determining the gradients are as follows: *a-c'*, motion downward of diastolic position of upper cone; *b-c*, motion upward of diastolic position of lower cone; *d-e*, position of the arterial wall during diastole determined by the balance between (1) arterial diastolic pressure, and (2) the compressing pressure and elastic resistance of the artery; *f-g*, motion downward of systolic position of upper cone; *g* or *g'-i*, position of arterial wall determined by balance between (1) systolic pressure and (2) decompressing pressure and elastic resistance of the arteries.

The amplitude of the volume oscillations with the pulse at any compression is determined by the distance between the diastolic and systolic volumes at that time. The maximal oscillation is at *b-h*.

to cause only a slight increase in the amplitude of the oscillations, the increase in the diastolic gradient due to the motion upward of the lower conical closure might cause the compression oscillations to decrease in amplitude before diastolic compression is reached. Further citations of conditions which might alter the form of the compression record are probably unnecessary, for it should be possible now to determine their effects by fitting them into the accompanying table and figure.

EXPERIMENTAL

Oscillation diagram. In none of our experiments with the circulation schema have the conditions corresponded exactly with any one of the sets discussed above. (1) Thus we have not attempted to com-

pletely eliminate compressibility of the compression space, but rather have experimented with compression chambers of relatively high and relatively low compressibilities. (2) In our experiments closure of the artery could occur only with a certain amount of stretching between the cannulae supporting it in the compression chamber. This factor was relatively greater with rubber tube than with artery, because of the greater bore of the former. (3) By reason of the attachment of the artery to these cannulae the effects of the upper conical closure of the *artery* are probably more important relatively than in estimations made under natural conditions. In this connection it should again be borne in mind that our rubber tubes were wider than the arteries; consequently under the same conditions the effects of the upper conical closure should be more striking in the case of the former; in effect, the compression chamber, although of constant length, was *relatively* longer when it contained artery than when it contained rubber tube. (4) Neither have any experiments been made, owing to practical difficulties, with wholly inextensible, though flexible tubing. Nevertheless, the conditions have been varied sufficiently to render the results of value as a check on the foregoing theoretical considerations.

Here it might be mentioned that a pressure of about 1 to 2 cm. of water was required to plumply fill the rubber tube used in these experiments. Beyond this point and within the range of pressure herein employed, the extensibility of the rubber tube was practically proportional to the distending pressures. Estimations of the extensibility of the artery were not made. Inasmuch as we were dealing with relaxed artery, it may be assumed (9) that its extensibility decreased as the internal pressure increased. It might also be mentioned here that a certain amount of pressure was necessary to just bring the middle segment of the walls into apposition with each other. With rubber tube this amounted to 3 to 4 cm. of water. On account of its much smaller bore it was rather difficult to determine the pressure necessary to just collapse the artery. It may, however, be assumed that it was approximately the same as in the case of the rubber tube.

With our apparatus it was convenient to begin each set of observations with low extra-arterial pressure and to increase this pressure in steps from below diastolic to above systolic pressure. The results have been plotted in two ways. (a) In order to make clear the general configuration of the records we have in a few instances plotted the oscillation *pressures* (ordinates) against the initial compressing pressures (abscissae). And (b) in order to facilitate a comparison of the present

records with the records as usually obtained in blood pressure observations, we have in all cases plotted the *amplitude* of the oscillations against the initial compressing pressures. It should be added here that

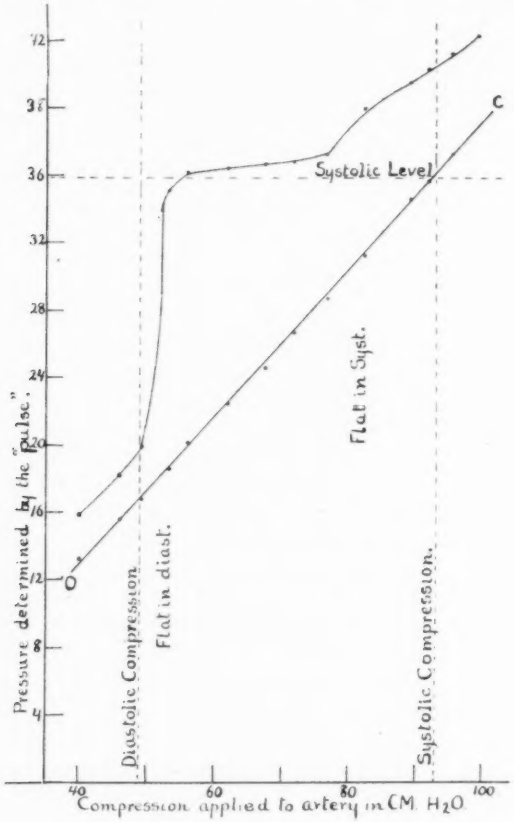

Fig. 5. Curve reconstructed from a record of the pressure changes in a compression chamber. Rubber tube; small compression space closed during diastole. The word "flat" in figures 5 to 16 when unqualified, is intended to mean that only the central part of the artery is just collapsed.

the deflections of the manometer connected with the compression chamber, which was calibrated in each experiment, were equal for equal increments of pressure within the range employed.

General configuration of records

Small compression chamber. The compression chamber made small by the means described above had an air space of about 10 cm. The configurations of the plots (figs. 5, 6, 7) made from experiments in which the rubber tube served as "artery" in this chamber bear a strik-

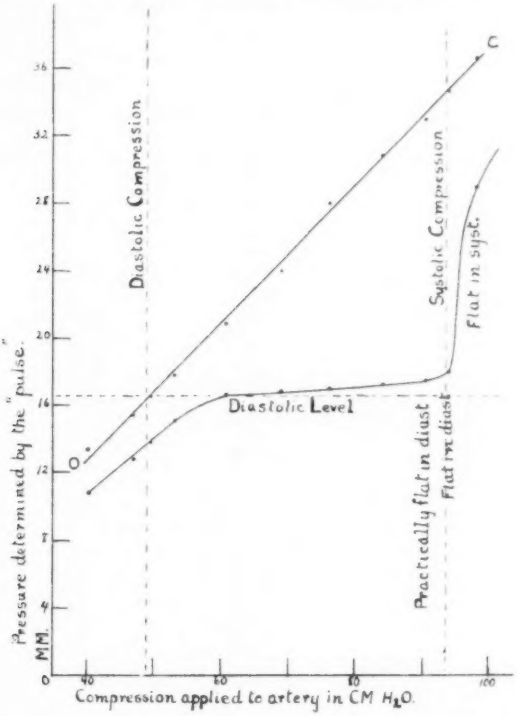

Fig. 6. Curve reconstructed from a record of the pressure changes in a compression chamber. Rubber tube; small compression space closed during systole.

ing resemblance to the diagram (fig. 2) constructed from theory. Thus when the compression is applied each time while the pulse is in its diastolic phase (fig. 5) the oscillations are built up on the gradient, *O C*, of the compressing pressure and have a configuration tending decidedly toward *D D'S* of figure 2. Again, when the compression is applied each time while the pulse is in its systolic phase (fig. 6), the oscillations

are built down from the gradient, $O\ C$, of the compressing pressure and have a configuration tending decidedly toward $D\ S'\ S$ of figure 2. And finally, figure 7, depicting the results obtained when the compression is applied while mean arterial pressure prevails, simulates in configuration $D\ M\ M'\ S$ of figure 2.

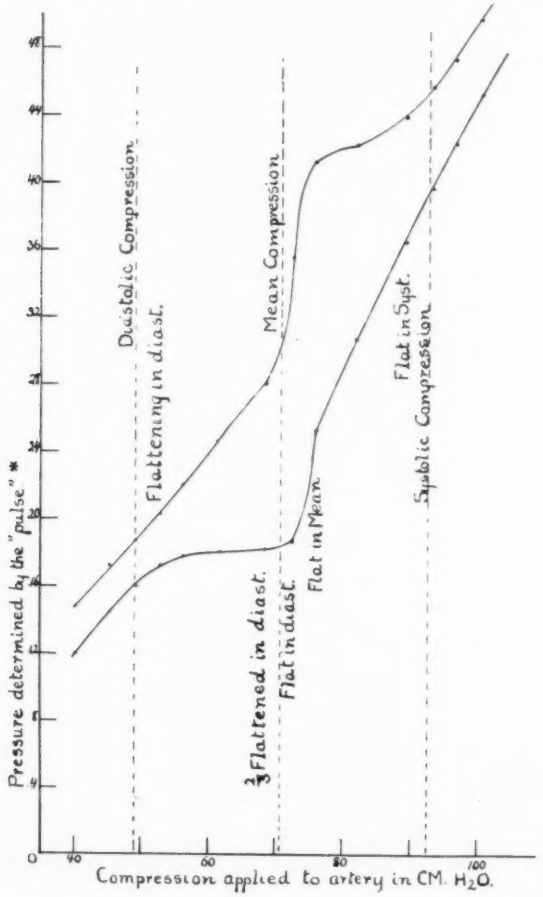

Fig. 7. Curve reconstructed from a record of the pressure changes in a compression chamber. Rubber tube; small compression space closed at mean arterial pressure. Only the relative ordinates of this curve are known.

We have not deemed it necessary to reproduce similar reconstructions of the records obtained from artery. Inspection of the reconstructions showing the oscillation amplitudes obtained in the case of artery (figs. 8 and 9) is all that is now needed to render obvious the general configurations of those records. The variations from the configuration of the curves obtained with rubber tube thus brought out are attributable to the fact that the volume of the artery relative to that of the compression space is less than that of the rubber tube; it therefore happens that the elastic arterial wall rather than the compression chamber supports the arterial pressure while the compression pressures are low.

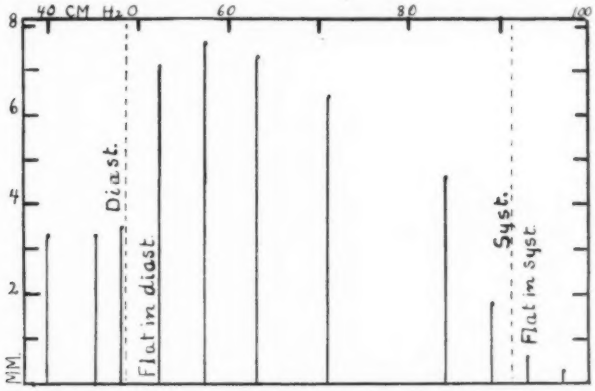

Fig. 8. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; small compression space closed during diastole. In figures 8 to 16 the abscissae indicate the compression pressure in cm. of water, the ordinates the amplitude of the compression pulse in mm.

When the compression is applied during the diastolic phase of the pulse (fig. 8) the oscillations, under compressions ranging within the pulse pressure, are high at first, and more or less uniform; they then decrease, though not to so low an amplitude as at corresponding pressures in the case of rubber tube. And when the compression is applied during systole (fig. 9) the oscillations in the diastolic-systolic range at first increase in amplitude and then decrease, though not to so low an amplitude as is obtained at corresponding pressures in the preceding case.

Large compression chamber. The air space of the compression chamber was then considerably increased, but in all tests of this set the com-

pressibility was adjusted so that the oscillations, whether from rubber tube or artery, were under similar conditions of approximately the same amplitude. Figures 10 and 11 illustrate the results obtained with the rubber tube in this larger chamber. In accordance with theory, it is seen that the amplitude of the oscillations increases slightly and more or less consistently through the diastolic-systolic range of compression. The results differ slightly, though in keeping with theory, according as the compression is applied during the diastolic (fig. 10) or the systolic (fig. 11) phases of the pulse.

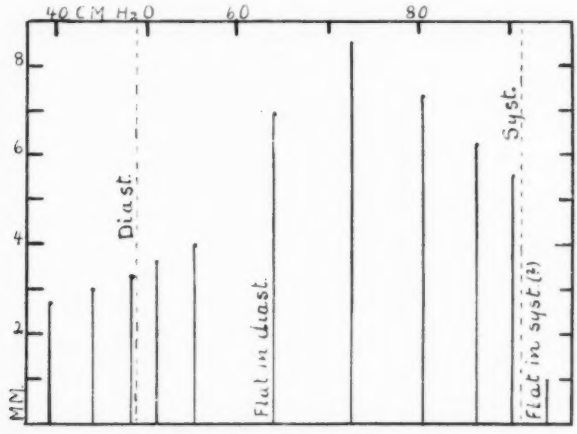

Fig. 9. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; small compression space closed during systole.

With artery, however, it is seen (figs. 12 and 13) that in the same range of compression the amplitude of the oscillations on the whole *decreases* in both cases, though again there are slight differences depending upon the phase of the pulse in which the compression is applied.

At first thought it might seem that this result is just the reverse of the one the theoretical considerations should lead us to anticipate; for the extensibility of artery is such as to have the effect of enhancing the decrease in the amplitude of the oscillations as seen in the diastolic-systolic range in the case of rubber tube. When, however, it is recalled that the bore of the artery is much smaller than that of the rubber tube, and that this has the effect of making the compression chamber longer

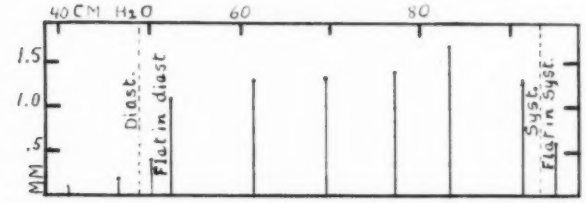

Fig. 10. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; large compression space closed during diastole.

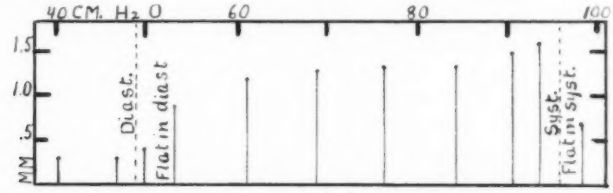

Fig. 11. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; large compression space closed during systole.

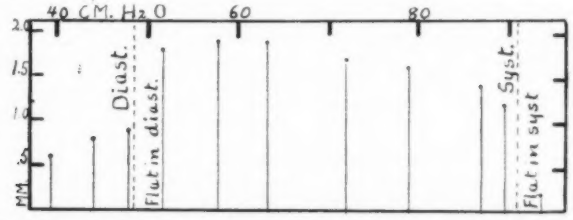

Fig. 12. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; large compression space closed during diastole.

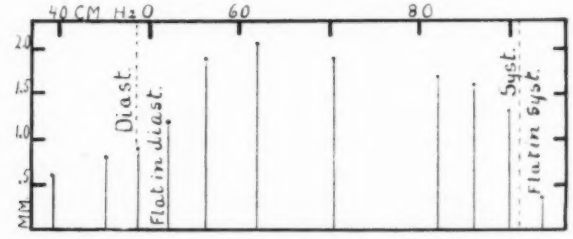

Fig. 13. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Artery; large compression space closed during systole.

relatively in the case of the artery, a satisfactory explanation of the results obtained with artery is found: the effect upon volume exerted through the upper conical closure is submerged by the effect upon volume exerted through the influence of the long bore of the artery (see below); though it is possible that in part this result is attributable also to an assumed smaller undistended bore and greater extensibility of the artery relative to the rubber tube.

Effect of length of artery upon the configuration of the oscillation record

In order to test the theoretical considerations that have to do with the influence of the length of the tube upon the configuration of the oscillation record, the oscillations obtained from a long (10.5 cm.) rubber tube have been compared with those from a short (5.4 cm.) rubber tube, under conditions as nearly alike as they could be made, excepting that the compressibility of the chamber was regulated so that the compression oscillations were of approximately the same height in both tests. These tests fully confirm the premises. With a longer tube the amplitude of oscillations within the diastolic-systolic range of compression changes very little; and the diminution in amplitude when the systolic level is reached is quite abrupt. With the shorter tube the results resemble closely those illustrated by figure 10: the oscillations increase in amplitude to the systolic level, and then decrease, though not so abruptly, as in the case of the longer tubes.

Critical oscillations

Turning now to the relation of the oscillation amplitudes and amplitude changes to the diastolic, mean and systolic arterial pressures, it is well first to recall that a pressure of from 3 to 4 cm. of water is necessary to bring the walls of the tubes into apposition with each other, and therefore that such compressing pressures as collapse the artery must be corrected by deducting 3 to 4 cm. from them.

Small compression chamber. Bearing this in mind and making allowance for the gaps between successive readings, it is seen (figs. 8 and 14) that when the compressing pressure is applied while diastolic pressure prevails the amplitude of the oscillations increases abruptly the instant the extra-arterial pressure exceeds the intra-arterial diastolic pressure, and that this increase is practically to the maximum. On the other hand, the decrease to the small oscillations that obtain when

the intra-arterial systolic pressure is exceeded takes place more or less gradually.

When the compression is applied while systolic pressure prevails in the system (figs. 9 and 15) the diastolic pressure, instead of being marked by an abrupt increase in oscillation amplitude, is indicated either by an amplitude of oscillation which begins to increase from a constant height (rubber tube) or which increases at a more rapid rate (artery); while the systolic pressure now is marked by a decrease in amplitude that is decidedly abrupt. Maximum oscillations are obtained either at systolic pressure (rubber tube) or considerably below systolic pressure (artery).

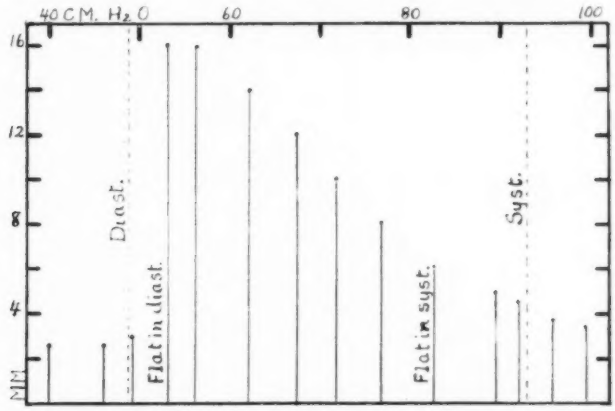

Fig. 14. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; small compression space closed during diastole.

When the compression is applied while mean arterial pressure prevails[5] the diastolic pressure (fig. 16) is indicated by a gradual increase in the amplitude of oscillations, systolic by a decrease in the rate of diminution; while maximal oscillations are obtained approximately at mean compression.

Large compression chamber. With the larger compression chamber, however (figs. 10, 11, 12 and 13), both the systolic and the diastolic pressures are invariably indicated by abrupt changes in the amplitude of the oscillations. The increase in the amplitude of the oscillations

[5] Done with rubber tube only.

at diastolic pressure is, with both rubber tube and artery, more abrupt when the compression is applied during diastole (figs. 10 and 12) than during systole (figs. 11 and 13); while under all circumstances it is more abrupt with artery than with rubber tube. Indeed, almost within the limit of error of measurement, the increase in amplitude when the compression is applied to artery during diastole is to maximum at once. And under all conditions, systolic pressure is marked by an abrupt decrease in the amplitude of oscillations. In accordance with theory the decrease is more marked with the rubber tube than with artery and when the compression is applied during systole than during diastole.

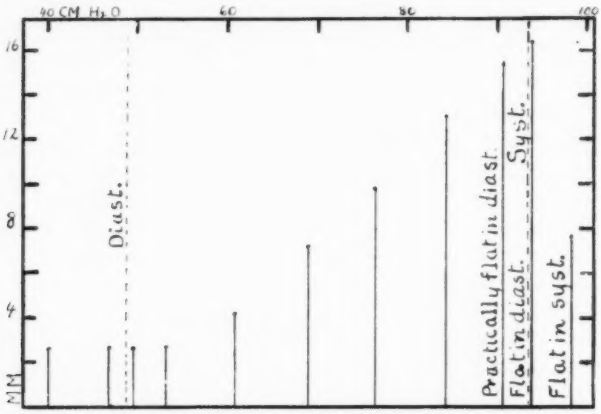

Fig. 15. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; small compression space closed during systole.

The influence on the oscillations of reducing the duration of the "pulse." A study of the influence on the oscillations of reducing the duration of the "pulse" has not been included in the present investigation. In our first investigation of this subject (3), however, a schema was used in which the pulses recurred at the rate of about 60 per minute. The records obtained at that time from rubber tubes made in the same way as were those employed in the present research, show that the oscillations in the systolic-diastolic range have a decided crescendo up to diastolic compression. In view of the fact that under the slow "pulse" of the present investigation the oscillation amplitude in the same range tended to decrease rather than increase, we feel justified

in concluding that the differences are attributable to the differences in the duration of the "pulses." This result, as a matter of fact, might have been predicted, for if time is a factor it must become increasingly potent as the compression decreases from the systolic to the diastolic level, since the time during which the effective pressure lasts increases throughout this period from a mere moment, under systolic compression, to the duration of the pulse itself under diastolic compression.

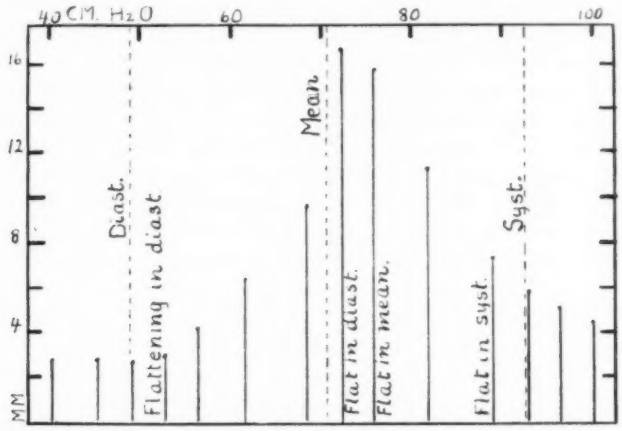

Fig. 16. Reconstruction from a record to show the amplitude of oscillations under all compressing pressures. Rubber tube; small compression space closed during mean compression.

Discussion of discrepancies in the literature

The marked influence exerted by the size of the compression space upon the relations obtaining between oscillation magnitude and arterial pressures in large measure accounts, we believe, for the diverse conclusions reached by different workers in this field. An attempt to account for all the differences recorded in the literature, in the light of our results, would however scarcely be worth while even if the articles contained the necessary data, and usually they do not. We do, however, desire to say a word with regard to the experiments of MacWilliam and Melvin (9), since these investigators, using a circulation schema similar to the one originally used by the author, have reached conclusions differing in some respects from those recorded by us. Thus while we found that the last of the series of maximal oscillations were

obtained at compression pressures that agreed fairly well with the arterial diastolic pressure, MacWilliam and Melvin find no such correspondence; in their experience maximal oscillations were obtained considerably above diastolic compression, while diastolic pressure seemed to be indicated rather by a sudden change in amplitude.

It will be noted that this is exactly the result obtained by us in the present series of experiments, when artery, under most circumstances, is pulsating in a comparatively small compression space. Now, although MacWilliam and Melvin do not give the dimensions of their compression chamber, we may be justified, by the fact that they used a considerable length of *large* artery, in assuming that it was small relatively. If so, the failure of the last maximal oscillations to agree with diastolic arterial pressure noted by them undoubtedly is partly referable to the great variation in the size of the artery (carotid of sheep and of ox) they employed, and also to the way in which they varied the volume of air in their compression chamber, to be inferred from the statement that "air was commonly used as the transmitting medium."

Motion of arterial walls in relation to critical oscillations and arterial pressures

The method we have used for the purpose of studying the compression oscillations offers a splendid opportunity for observing the relation of the swing of the arterial wall to the critical compression oscillations. These observations have shown (see the notes inscribed on figs. 5 to 16) that *the* maximal oscillation is obtained under all circumstances at a compression within the range that brings the arterial walls into apposition, it matters not what the relation of the maximal oscillation to the arterial pressures may be. This observation is in striking contrast to that of MacWilliam and Melvin who state that maximal oscillations develop when normal distensible artery[6] is compressed only to the "half-flattened" state.

[6] MacWilliam and Melvin, however, find that with non-distensible tube or artery maximum oscillation occurs when the compressing pressure causes complete or almost complete flattening during diastole. Is it possible that their non-distensible tubes and arteries were smaller than their distensible tube and that this difference in the behavior of the two types of tube is referable rather to differences in the relative compressibility of the compression space thus determined and to differences in the amount of fluid, i.e., the time, required to fill the tubes, than to differences in extensibility of the tubes?

While MacWilliam and Melvin surmise that the discrepancy is referable to differences in the time factors of the "pulses" and possibly in the volume of "blood" moved under the compression in the two sets of experiments, they nevertheless seem to be satisfied that their results and the explanation they give to account for them are directly applicable to conditions that obtain in blood pressure observations in man. It can easily be shown, however, that this attitude is wholly untenable.

The pulse in the circulation schema used by me (3) was developed by a rotating stopcock which put the "artery" into communication with the head of pressure during one-third of each revolution. We showed that the wave thus developed resembled the arterial pulse fairly closely. MacWilliam and Melvin presumably produced their pulse by an electro-magnetic interrupter which "temporarily" occluded the tube leading from the pressure bottle usually 60 times a minute (13). These investigators fail to give the duration of the temporary occlusion, nor do they say anything in regard to the configuration of the pulse thus obtained. An examination of their curves with a magnifying glass makes it obvious, however, that they were not dealing with a pulse of the usual form but rather with a very complicated one in which the pressure was low for a very brief period and high for a comparatively long period. Now a pulse of this form accounts perfectly for the fact that in their experiments the "artery" did not flatten under a compression that exceeded the diastolic pressure.

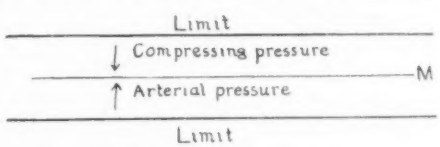

Fig. 17. Diagram to illustrate the influence of the configuration of the pulse upon the movements of the arterial wall.

For the purpose of making this clear we will regard the arterial wall as a membrane, *M* (fig. 17) free to move between two opposing pressures, the arterial pressure acting from one side, the compressing pressure from the other. The movements of this membrane are limited, on one side by contact with the opposite wall (that is by complete collapse) and upon the other by the distended position of the wall. Under these circumstances the membrane would be held against one wall as long as the excess of pressure was in that direction, and would move completely across the space to the other wall the instant the excess of pressure was in the other direction. This would be the type of motion

as long as the compressing pressure lay within the pulsatile range of arterial pressure. But let us now assume that this membrane is so damped by the fluid around it, or, if you will, upon one side of it, and that the phases of the pulse are so brief that the disturbances of the equilibrium determined by the pulse do not last long enough to carry the membrane to these limits. Under such circumstances, which are indeed the circumstances obtaining in an artificial circulation schema and possibly also in actual estimations of the blood pressure, the position assumed by the membrane would in addition depend upon the relation of the arithmetic mean of the maximum and minimum pressures of the pulse to the geometric mean of the pulse area. If the geometric mean lies to the systolic side of the arithmetic mean, that is, if the pressure is high for a longer period than it is low, the membrane will not have time during the period of low pressure to reach the lower limiting side and hence will oscillate from the upper wall for a part of the period during which the compressing pressure lies within the range of the pulse pressure: the artery would then close no further than to the "half-flattened" position of MacWilliam and Melvin. If, on the other hand, the geometric mean lies to the diastolic side of the arithmetic mean, which is true of all arterial pulses, the membrane, within the same range of pressure, would oscillate from the lower wall of the diagram, but might not attain the upper wall. That is to say, throughout the pulse pressure range of compression the artery would be completely collapsed during a part of each pulse cycle; it would cease to resume the collapsed position at some time in each pulse only when the compressing pressure became less than the diastolic pressure.

When, in the present experiments, the compressibility of the compression space is small, to return to a discussion of our results, maximal oscillations under all circumstances (figs. 8, 9, 14, 15, and 16) coincide more or less exactly with the compression that *just* flattens the tube and if, in addition, the compression has been applied during the diastolic phase of the pulse, this occurs more or less exactly at diastolic compression (figs. 8 and 14).

When the compression space is large (figs. 10, 11, 12 and 13) the artery is *just* flattened exactly at diastolic compression under all circumstances. Reading the figures backward, last flattening is always immediately followed by a sudden diminution in the amplitude of oscillations, and when the compression is applied to *artery* during diastole (fig. 12), last flattening and *last* maximal oscillation agree exactly with diastolic compression.

EXPERIMENTS ON ARTERIES IN SITU

Methods in general

The methods employed in the present animal experiments will be fully described elsewhere. Here it will suffice to say that the compression was applied to the dog's femoral artery through our arteriograph (3). The compression chamber usually had a capacity of from 0.5 to 1 L., approximately. The pressures in it were recorded photographically by a Frank mirror capsule connected with the compression space either directly, or indirectly through the author's sphygmomanometer. The records were made both by the method of continuous escapement, when the recording tambour was provided with a pinhole opening, and by the method of intermittent escapement, when the tambour system was completely closed while the records were in the process of making. The arterial pressure was not recorded but the sounds heard in the arteries below the compression chamber were signaled and often recorded also. The pulse peripheral to the compression chamber was also recorded by a Frank mirror capsule. The relation the sounds bear to the oscillations will form the subject of another communication; for the present it will be arbitrarily assumed that the appearance of sound indicates the systolic pressure and that the dulling of the sounds at the end of the 3d phase is an index to the diastolic pressure. The conditions obtaining in the animal experiments resemble those of a long artery in a large compression chamber.

RESULTS

General configuration of the compression records

It was not expected that these methods would shed any new light on the general configuration of the compression record as a whole. It is, however, worth while noting that the records obtained with the Frank capsule by both the continuous and intermittent escapement methods have exactly the same general outlines as those that are obtained with the usual apparatus. The records are too long for reproduction here but their contours may be judged by the measurements of the oscillation amplitudes of a number of typical records which have been collected in Tables II and III. It is there seen that the very gradual increase in amplitude at first recorded during decompression shows a decided acceleration, beginning about 5 to 15 mm. Hg. above the level at which the first sound is heard, the largest absolute increase,

TABLE II

Analysis of photographic records of blood pressure estimations by the method of continuous escapement (tambour pin hole open) from the bare artery of the dog.

DOG NO. 10			DOG NO. 13		
No. of pulse*	Oscillation amplitude	Time to peripheral pulse	No. of pulse‡	Oscillation amplitude	Time to peripheral pulse
	mm.	*sec.*†		*mm.*	*sec.*§
1	4.5		5	2.0	
2	6.5		9	2.0+	
3	7.5		13	2.0	
4	6.5		17	2.5	
5	10.5		21	3.0	
6	9.0		25	5.5	
7	11.0		29	5.5	
8	12.5		33	5.0	
9	11.5		37	6.5	
[1]10	16.0	0.071	41	14.0	
11	13.5		[1]42	17.0	
12	16.0	0.063	45	21.0	0.085
13	17.0	0.071	46	19.0	0.102
14	16.5	0.071	49	21.0	0.099
15	18.5	0.060	52	25.0	0.074
16	18.0	0.079	55	25.0	0.076
17	19.5	0.059	59	26.0	0.063
18	20.0	0.056	64	26.0	0.063
19	19.0	0.066	69	28.0	0.063
20	20.5	0.053	73	28.0	0.048
21	20.5	0.054	77	27.0	0.054
22	21.0	0.048	81	28.0+	0.05
23	22.0	0.048	85	29.5	0.046
24	21.0	0.057	89	30.0	0.04
25	22.5	0.043	93	30.0	0.038
26	22.0	0.043	97	31.0	0.038
27	22.0	0.046	101	33.0	0.043
28	23.0	0.038	105	36.0	0.037
29	22.0	0.043	109	39.0	0.031
30	23.0	0.037	113	40.5	0.036
31	23.0	0.035	114	40.0−	0.038
32	23.0	0.043	115	40.5	0.038
33	23.5	0.032	116	41.0	0.034
34	23.5	0.034	117	40.0	0.038
35	24.0	0.035	118	40.0	0.032
36	24.0	0.028	119	41.0	0.037
37	24.0	0.038	[1]120	40.0	0.031
38	25.0	0.026	121	40.0−	0.032

TABLE II—Continued

DOG NO. 10			DOG NO. 13		
No. of pulse*	Oscillation amplitude	Time to peripheral pulse	No. of pulse‡	Oscillation amplitude	Time to peripheral pulse
	mm.	*sec.*†		*mm.*	*sec.*§
39	25.5	0.028	122	41.0	0.031
40	26.0	0.026	123	39.5	0.027
41	26.0	0.025	124	38.5	0 031
42	27.0	0.028	125	38.0	0.025
43	26.0	0.025	126	37.0	0.029
44	26.5	0.024	[4]127	34.0	0.021
45	23.0	0.024	128	33.5	0.029
46	23.5	0.019	129	32.5	0.024
47	24.5	0.021	130	30.5	0.024
[2]48	19.0	0.013	131	28.0	
49	18.5	0.012	132	30.0	0.018
50	14.0	0.013	133	27.5	0.02
51	14.5	0.010	136	25.5	0.016+
52	15.0	0.012	138	24.5	0.016
53	13.0	0.088			
54	13.0	0.088			
55	11.0	0.073			
56	11.0	0.059			
57	11.5	0.044			
58	10.0	0.029			
59	10.0	0.029			
60	9.5	0.044			
61	9.0	0.029			
62	9.0	0.029			

* The mean rate of pressure decrease is 2.2 mm. Hg. per pulse; the rate in the vicinity of systolic pressure and of diastolic pressure is approximately 2.5 + mm. Hg. and 1.9 − mm. Hg. per pulse, respectively.

† The time was recorded in seconds. The paper, however, moved very uniformly at the rate of 68 mm. per second. The figures given in this column are therefore approximately correct.

‡ The rate of fall of pressure in the vicinity of pulse No. 5 is roughly 1 mm. Hg. per pulse; in the vicinity of pulse No. 123 it has decreased to approximately 0.5 mm. Hg. per pulse.

§ The time is recorded in fifths of seconds. The speed of the paper was not absolutely uniform but varied regularly between 16 and 20 mm. per 0.2 second. 1 mm. may therefore be regarded as roughly equivalent to 0.0112 seconds.

[1] First sound.

[2] Sound fainter.

[3] Possibly fainter.

[4] Certainly fainter.

TABLE III

Analysis of photographic records of the blood pressure estimations by the method of intermittent escapement (tambour pin-hole closed) from the bare artery of the dog.

DOG NO. 14			DOG NO. 15			DOG NO. 19		
Compressing pressure	Oscillation amplitude	Time to peripheral pulse	Compressing pressure	Oscillation amplitude	Time to peripheral pulse	Compressing pressure	Oscillation amplitude	Time to peripheral pulse
mm. hg.	*mm.*	*sec.*	*mm. hg.*	*mm.*	*sec.*	*mm. hg.*	*mm.*	*sec.*
160	1-1.5		155	1		170	1.0	
155	1-1.5		150	1+		165	1.3	
150†	1.5-2.5		145	1+		160	1.5	
145	3.0		140	2-5.5		155	2-4.5	
140	6.5-8.5		135*	6-13	0.083-0.094	150*	5-8	
135	6.5-9.0		130	10-15	0.072-0.078	145‡	7-10	
130*	11-14.5	0.079	125	17-22	0.068	140	10-11	0.054
125	15-15.5	0.079	120	20-23	0.034	135	11-11.3	0.043+
120	16-18	0.055	114	22-24	0.034	130	12+	0.046
110	18-18.5	0.049-0.055	110	24-25.5	0.044	125	12.5	.036
105	17.5-18	0.049-0.051	105	26-27	0.042	120	13.0-13.5	0.034
100	18.0	0.041	100	26-27.5	0.038-0.042	115	13.5-13.7	0.027
95	19.5-20	0.031-0.035	95	27-27.5	0.038	110	14.0	0.0238
90†	16-18	0.017-0.021	85	28.5-29	0.038	105	12.5	0.0222
85	12-12.5	0.015-0.017	80†	24-25	0.022-0.028	100†	10.0	0.0155
80	8.5-9.0	0.009-0.011	75	16-20	0.028	95	8.0	0.014-
			70	16.0	0.02-0.016	90	6.0	0.0126
			60	11.5	§	85	5.5	0.0126-0.0142
						80	5.2	0.0142

* First sound. † Sounds fainter. ‡ Pulse first becomes visible. § Pulse wave not clear.

however, coinciding, within the limit of error of the method, with the very pulse that determines the first sound. Then the increase in amplitude becomes more and more gradual. In about half of the records this gradual increase either continues, or the oscillations attain and maintain a uniform height, until an abrupt diminution in amplitude is registered; in the other half a gradual though slight diminution in amplitude is seen to begin a variable time before an abrupt diminution occurs. The dulling of the sounds always coincides *exactly* with the sudden diminution in amplitude. The general configuration of the records therefore agrees perfectly with the theoretical consideration.

Volume pulse of the compressed artery

Inasmuch as the theoretical development of this subject has indicated that the volume changes of the compressed artery are probably of prime significance in the production of compression oscillations, an

attempt has been made to gain some specific knowledge with regard to the actual volume changes an artery experiences while it is being gradually decompressed.

To obtain this information the following method has been employed. The arteriograph and a horizontal glass tube 3 mm. in bore extending from it to an air chamber of about 1 liter capacity, were filled with water to the total exclusion of air to a chosen position in the tube. The meniscus of the water in the tube therefore moved to and fro as the volume of the artery changed with the pulse and with the pressure exerted back upon it by the air in the air chamber beyond. The motion of the meniscus was photographed by causing its image to fall upon the slit of a photo-registering apparatus. The tube was calibrated by measuring the distance the image of the meniscus moved when known amounts of water were allowed to flow into it. Inasmuch as the volume of water moved by the pulse in this apparatus is quite considerable the position attained by the meniscus during systole is probably subject to some error. Nevertheless, the general accuracy of the method is indicated by the fact that the bore of the artery derived by calculation from the volume change as given by the record and the known length of the artery compressed, shows a surprisingly close agreement with the actual bore of the artery. Thus in the experiment here used for purposes of illustration the maximum increase in volume from the zero level is something over 0.6325 cc. (see Table III). The effective length of the arteriograph, that is, the length of artery compressed, is something over 5 cm. Therefore the maximum bore of the artery is (Vol. $= \pi r^2 \times L$; or $r = \sqrt{\frac{.6325}{\pi \times 5}} = 0.2$ cm.) 4 mm. How nearly correct this result is may be judged by the fact that the orifices of the arteriograph measure 5 mm. in diameter and that while adjusting this instrument it was always moved up the artery until the latter just filled the orifices without actually being pressed upon.

The beginning of the 1st phase and the beginning of the 4th phase of the Korotkoff sounds were signaled upon the same record. It should be added that for reasons to be considered in another connection the early first phase sounds may not be as distinct as usual, and may even be missed, when the compression tube is filled with water. Therefore, the first signal in these experiments probably was often later by a few pulse waves than it would otherwise have been.

We are reproducing here one of the records of the volume changes of the compressed artery (fig. 18), greatly reduced in size. The first sig-

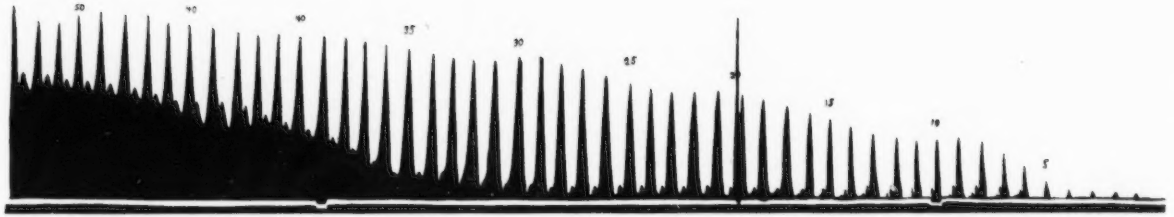

Fig. 18. Record of the volume changes of an artery in the compression chamber while it is being gradually decomposed. Reduced to $\frac{1}{4}$ size. The record reads from right to left. On the original an elevation of 1 cm. equals a volume change of 0.055 cc., approximately.

nal, for the reason just given, is undoubtedly several pulse beats late. We will assume it should have signaled the 5th pulse of the record. The 2d signal, allowing for reaction time, signals the 38th pulse. The record is analyzed in Table IV. If the wave-like fluctuations, which probably are caused by respiratory blood pressure changes, are disregarded, the resemblance of the contours of this record to those of figure 4 is seen to be very close. Thus, the basal or diastolic, volume rises very gradually, during 31 pulses, to within 6 pulses of the diastolic pressure (beginning of the 4th phase sounds). With the 32d pulse it begins to increase somewhat more rapidly, though still relatively slowly, until diastolic compression is attained (37th pulse), when a much more rapid increase in the basal volume begins—an increase which, judging by the new curve it follows, must be due to the entrance of a wholly new factor. Now, if we assume that the first gradual increase in the diastolic volume is due to the extension downward of the upper conical closure of the artery,[7] we should expect the lower cone to add at least a like amount of blood when it begins to manifest itself. This latter addition to the volume of the blood in the compression tube should occur rather rapidly when the peripheral artery begins to fill rapidly, that is, when the compression approaches the diastolic level, and the effects at the two cones should then summate. It is therefore interesting, to say the least, that the first slow rise involves an addition of 0.0577 cc. of blood, while the total rise at diastolic compression (37th pulse) amounts to 0.1402 cc., an increase of about $2\frac{1}{2}$ volumes; the relation of the two volumes to each other is quite in keeping with the premises. And it is probably more than a mere coincidence that the volume obtaining during systole just before the pulse presumably breaks through (5th pulse—0.0825 cc.), i.e., when the upper cone is manifesting its maximum effect upon the volume of the compressed artery—is roughly one-half of the total increase in volume (0.1402 cc.) attained during diastole just before the lower conical closure is obliterated by the opening out of the whole length of the artery (37th pulse). It is, however, realized that this relation is influenced somewhat by the level of the compressing pressure at which the record happens to start and by the resistance the blood happens to experience in flowing out of the artery into the veins.

Finally it seems justifiable to conclude that the new factor that has

[7] This is probably the most important factor; a certain amount of the increase may be attributable to the opening out of the artery at points where it is sharply creased by the compression.

TABLE IV

Analysis of a record (fig. 18) of the volume changes in a compressed artery. Method of continuous escapement. Dog No. 21

NO. OF PULSE*	ELEVATION AT END OF DIASTOLE	VOLUME AT END OF DIASTOLE	ELEVATION AT CREST OF PULSE	VOLUME AT CREST OF PULSE	AMPLITUDE OF OSCILLATIONS	VOLUME OSCILLATION WITH ANACROTIC LIMB
	mm.	*cc.*	*mm.*	*cc.*	*mm.*	*cc.*
1	0?	0?	9.0	0.0495	9?	0.0495
2	0?	0?	7.0	0.0385	7?	0.0385
3	0?	0?	8.0	0.044	8?	0.044
4	1.5?	0.0082	11.5	0.0632	10.0?	0.055
5	3?	0.0165	17.0	0.0935	14?	0.077
6	4?	0.022	25.0	0.1375	21?	0.1155
7	5.5	0.0302	34.0	0.1875	28.5	0.1567
8	6.0	0.033	41.0	0.2255	35.0	0.1925
9†	6.0	0.033	43.0	0.2365	37.0	0.2035
10	6.0	0.033	43.0	0.2365	37.0	0.2035
11	6.0	0.033	41.0	0.2255	35.0	0.1925
12	6.5	0.0357	44.0	0.242	37.5	0.2062
13	6.0	0.033	46.0	0.252	40.0	0.220
14	6.5	0.0357	50.0	0.275	43.5	0.2392
15	6.5	0.0357	55.0	0.3025	48.5	0.2667
16	7.0	0.0385	58.0	0.3190	51.0	0.2805
17	7.0	0.0385	62.5	0.3437	55.5	0.3025
18	7.5	0.0412	67.0	0.3685	59.5	0.3272
19	8.0	0.044	70.0	0.3850	62.0	0.3410
20	——	——	——	——	——	——
21	8.5	0.0467	72.0	0.3960	63.5	0.3492
22	9.0	0.0495	71.0	0.3905	62.0	0.3410
23	9.5	0.0522	71.5	0.3932	62.0	0.3410
24	9.5	0.0522	72.5	0.3987	63	0.3465
25	9.0	0.0495	76.0	0.4180	67	0.3685
26	8.5	0.0467	82.0	0.4510	73.5	0.4042
27	8.5	0.0467	85.0	0.4675	76.5	0.4207
28	9.0	0.0495	88.5	0.4876	79.5	0.4372
29	9.5	0.0522	91.5	0.5032	82.0	0.4510
30	10.0	0.055	92.5	0.5087	82.5	0.4537
31	10.5	0.0577	92.0	0.5060	81.5	0.4482
32	12.5	0.0687	91.0	0.5005	78.5	0.4317
33	14.0	0.077	91.5	0.5032	77.5	0.4262
34	16.0	0.088	93.5	0.5142	77.5	0.4262
35	19.0	0.1045	96.5	0.5307	77.5	0.4262
36	22.0	0.121	100.0	0.5500	78.0	0.4290
37	25.5	0.1402	104.0	0.572	78.5	0.4317
38‡	34.5	0.1897	106.5	0.5857	72.0	0.3960
39	38.5	0.2117	107.0	0.5885	68.5	0.3767

TABLE IV—Continued

NO. OF PULSE*	ELEVATION AT END OF DIASTOLE	VOLUME AT END OF DIASTOLE	ELEVATION AT CREST OF PULSE	VOLUME AT CREST OF PULSE	AMPLITUDE OF OSCILLATIONS	VOLUME OSCILLATION WITH ANACROTIC LIMB
	mm.	*cc.*	*mm.*	*cc.*	*mm.*	*cc.*
40	45.0	0.2475	107.0	0.5885	62.0	0.3410
41	49.0	0.2695	107.0	0.5885	58.0	0.3190
42	52.5	0.2887	108.0	0.5940	55.5	0.3052
43	57.0	0.3135	109.0	0.5995	52.0	0.2860
44	60.0	0.33	110.0	0.605	50.0	0.275
45	62.0	0.3410	112.5	0.6187	49.5	0.2723
46	63.0	0.3465	114.0	0.627	51.0	0.2805
47	64.0	0.3520	115.0	0.6325	51.0	0.2805
48	66.0	0.363	115+	0.6325	49+	0.2695
49	67.0	0.3685	115+	0.6325	48+	0.2640
50	69.0	0.3795	115+	0.6325	46+	0.2530
51	71.0	0.3905	114.5	0.6298	43.5	0.2392
52	71.5	0.3932	115.0	0.6325	43.5	0.2392
53	72.5	0.3987	115.0	0.6325	42.5+	0.2337

* Pulse rate is 174. The rate of fall of pressure averages 1.4 mm. Hg. per pulse; it is therefore somewhat faster in the vicinity of the first signal, and somewhat slower in the vicinity of the second signal.

† First sound.

‡ Sound fainter.

its inception with the very first of the 4th phase sounds, and which manifests itself in the form of a greatly accelerated increase in the volume of blood in the compression chamber, is the failure of the arterial walls to meet during diastole. The potency of this new factor is indicated by the relatively large volume change with which it is ushered in. Thus whereas during the 37 pulses that precede its appearance the basal volume of the artery increases 0.1402 cc., the volume increase with the very first pulse of the new gradient alone amounts to 0.0495 cc. It should be added that the record as reproduced here does not give an accurate conception of the true gradients of the volume changes. The record was made by the method of continuous escapement, in which the pressure on the artery falls at a constantly diminishing rate. Therefore the actual gradients are somewhat steeper in the latter parts of the record relative to those of the earlier parts. It is not necessary to correct the record for this effect because the correction would only serve to exaggerate the significant features of the experiment as shown in the record.

The systolic volume at first is small and increases very slowly, though in the case of figure 18 the initial compression was not quite high enough to bring out this feature clearly. The volume then increases, very rapidly at first (5th to 8th pulses inclusive), and then more and more slowly to the end of the record. These volume changes resemble so closely those drawn into figure 4 as to render further discussion unnecessary; their probable causes can easily be deduced by bearing in mind the discussion of the subject in connection with the development of that figure.

The maximum volume oscillation was registered with the 30th pulse, though it is quite probable that if the even course of the record had not been disturbed at this time by a respiratory wave of blood pressure, it would have been recorded somewhat later. In any event, it is the 2d gradient of the base line, which, we have inferred, is due mainly to the extension of the lower conical closure, that causes the diastolic volume to rise more raipdly than the systolic volume and so determines the appearance of the maximum oscillation at this time. As a matter of fact, however, the reduction of the volume oscillations from this, the 30th, pulse to the last pulse of the 3d phase (37th) is so slight, being only a little over 4 per cent, as to be almost negligible, especially in view of the fact that the diminution in volume with the very next pulse amounts to about 8.3 per cent.

Importance of the lower conical closure as determined by occlusion of the artery

That the 2d gradient of the diastolic volume, as seen in figures 4 and 18, might be determined by the growth of the lower conical closure is supported by some experiments in which the effect upon the compression pulse of occluding the artery some distance below the compression chamber was studied. In these experiments the compression oscillations were recorded by a Frank mirror capsule attached to the author's sphygmomanometer. The method of intermittent escapement was used. At each of the successive decrements a record was made first with the artery open, then occluded and finally open again. The data collected in Table V show that occlusion of the artery at compressions lying below the systolic pressure determine an elevation of the base line. This, it will be recalled, could be due only to an increase in the volume of the artery in the compression chamber. Such an increase in the basal volume might be caused either by a general

rise of blood pressure, which would manifest its effect through the upper conical closure, or by an increase in the peripheral pressure which would manifest itself mainly through an extension upward to the lower conical closure. That the first factor is, however, not of any considerable significance, is indicated by the observation (a) that the elevation of the base line is almost as marked, though much more gradual, in the higher ranges of systolic-diastolic compression as in the lower ranges, and (b) that the elevation of the base line is very slight when the artery is closed while under compressions lying below the diastolic pressure; for occlusion under the former conditions could cause little if any rise of the central pressure, the artery already being practically shut at that time by the compression itself, yet a considerable

TABLE V

Showing the rate of elevation of the base line when the artery is occluded while recording the oscillations in the compression chamber by the method of intermittent escapement (tambour hole closed)

COMPRESSION PRESSURE IN TERMS OF SOUND PHASES	MAXIMUM ELEVATION	NO. OF PULSES ELAPSING
	mm.	
1st Phase	3−	6−
2d Phase	3−	4
Late 2d Phase	3−	2
3d Phase	3+	2
Last 3d Phase	3++	1
4th Phase	1	1

elevation of the base line occurs. On the other hand, occlusion under the latter conditions probably does increase the central pressure, but it causes only a slight elevation of the base line. Still it is probable that the effect of occlusion of the artery upon the diastolic volume is not manifested wholly through the lower conical closure, for if it were, it would seem that the effect should diminish somewhat with decompressions within the systolic-diastolic range, and it does not. Furthermore, the elevation of the base line in this experiment amounts to 20 per cent of the amplitude of the maximum oscillation. In the experiment recorded in Table IV the 2d basal gradient lifts the base line (0.1402–0.0687) 0.0715 cc., or 16 per cent of the amplitude of the maximum oscillation. These figures are near enough alike, considering the fact that they are taken from different experiments, to signify that the

additional factor in the occlusion experiments determining an increase of volume is not very considerable.[8]

The experiment also shows, by the much more rapid distension of the artery when it is occluded in the later systolic-diastolic stages, that under usual conditions the influence of the lower cone must be far more significant then than during the earlier stages. The peripheral resistance, of course, will be of considerable significance in this connection.

Form of the artery in relation to the maximal oscillation

As shown by volume records. Special attention is directed to the fact that the maximal oscillation (30th pulse of fig. 18) is not recorded while the artery is in the "half-flattened" position during diastole. As a matter of fact the diastolic volume at this time has increased only (0.0577 – 0.033) 0.0247 cc., above the diastolic volume under systolic compression. This is less than one-sixth of the volume increase during the whole of the systolic-diastolic range, and considerably less than the volume increase (over 0.04 cc.) associated with each pulse at a time when the artery is completely collapsed during the entire pulse cycle (1st to 5th pulses). Indeed, the elevation of the diastolic volume, as we have already indicated, is attributable rather to the extension of the upper conical closure than to any opening of the middle region of the compressed artery. The records thus obtained from animals, therefore, completely confirm the results obtained under the ideal conditions supplied by a properly constructed and properly operated circulation schema. In view of the fact that it is possible, on the basis of the configuration of the pulse they used, to account satisfactorily for the finding of MacWilliam and Melvin to the effect that the artery in their circulation schema oscillated from the "half-flattened" position rather than from the flattened position while maximum oscillations were recording, there is no longer any good reason for doubting the earlier observations on this subject. Indeed, there is now every reason for holding, as we have held in the past, that under the conditions that obtain in practical sphygmomanometry, barring possibly a loss in the transmission of the pressure to the artery, the arterial walls continue to meet during diastole until the compressing pressure falls below the diastolic pressure.

[8] The additional factor will be considered in a subsequent paper.

As indicated by the time elasping between the beginning of the compression and peripheral pulses. If the compression pulse and the pulse in the artery just below the compression chamber be recorded simultaneously by Frank capsules it is found that with diminishing compression pressure the interval between the beginning of the compression pulse and of the peripheral pulse diminishes more or less steadily and relatively rapidly until the 3d phase sounds cease (see Tables II and III). Beginning with the 4th phase sounds the interval remains fairly constant or continues to decrease, though at a very much slower rate. Between the last of the 3d phase and the first of the 4th phase pulses this diminution in the time interval is the greatest that occurs in this particular region of the record. It seems fair to assume in explanation of this phenomenon that as long as the time interval is diminishing at a relatively rapid rate the artery is opening from the collapsed state earlier and earlier in the pulse cycle; that the marked diminution in the interval usually observed with the beginning of the 4th phase sounds marks the moment when the walls first fail to meet during diastole; and finally that the slower subsequent diminution in the interval in part at least is an expression of the increase in the coefficient of elasticity of the artery as diminishing compression permits the tension peripherally to increase.

Observations bearing on the time the arterial walls first begin to separate during decompression

A series of observations was also made in an effort to ascertain the moment, in relation to the compressing pressure and arterial pressure, the arterial walls first begin to open out under a compression that is falling from above the systolic arterial pressure. It was found by trial that the following was the most delicate method for making these observations. The bare femoral artery was closed by compressing it in the arteriograph. A long, straight, narrow glass tube was then inserted into the artery some distance below the arteriograph and by momentarily lowering the pressure on the artery blood was permitted to flow under the obstruction until it appeared in the tube. Then, beginning well above the systolic level, the compressing pressure was permitted to fall *very slowly* and the compressing pressure was read by an assistant as the observer, who could not see the manometer, announced (*a*) when the blood began to move in the tube, (*b*) when this progression first became distinctly pulsatile, (*c*) when it became decidedly pulsatile and (*d*) when the first sound was heard. After each observa-

tion the tube was flushed out with carbonate solution in order to prevent clotting. Somewhat greater accuracy would have been attained if the compressing pressure had been recorded and the observer had signaled the readings on the same record. The fact, however, that the compressing pressure was falling very slowly really made that method unnecessary.

TABLE VI

Showing the character of the blood flow through the compressed artery during the early stages of decompression

1 DOG NO.	2 BLOOD FLOWS	3 PULSE APPEARS	4 SOUND APPEARS*	5 DIFFERENCE 2–4
	mm. Hg.	*mm. Hg.*	*mm. Hg.*	*mm. Hg.*
15	142	134	128	14
	142	134	132	10
	141	136	129	12
	142	136	132	10
	142	137	132	10
	140	134	130	10
	146	138	136	10
Average....	.....	.....	.....	11
16	114	110	106	8
	114	111	108	6
Average....	.....	.....	.....	7
17	148	146	140	8
	148	146	132+	16
	148	145	136	12
	148	144	134	14
Average....	.....	.....	.....	12.5

* Also brusque pulse.

It is seen in the Table (VI) giving the results of these observations that the brusque pulsatile progression of the blood and the first sound invariably make their appearance simultaneously. It should be added that the change in the character of the pulsatile progression at this time is sudden and unmistakable. They appear on the average from 7 to 12 mm. of mercury below the compression under which the column of blood first begins to move. The earliest obvious pulse appears under intermediate compressions.[9] The figures giving the appearance

[9] This pulse must be exceedingly feeble, for only rarely can it be felt; and our method of recording the pulse, which was probably not quite as delicate as the finger, has never brought it out.

of the pulse and of sound vary considerably and the latter more than the former, relative to those indicating the pressure under which the blood begins to flow. This, we believe, is not due to any differences in the sharpness of the criteria, but rather to the fact that pulsation and the sounds are more apt to show the effects of the pressure changes associated with each individual pulse than is the beginning of flow, which probably is determined by small accretions from many pulses.

Be this as it may, the essential facts brought out by these observations are that the blood begins to pass through the artery, though exceedingly slowly, under a compression that exceeds the level at which the first sounds are heard by from 7 to 12 mm. of mercury; and that in the latter part of this stage the flow may become slightly pulsatile. It might be mentioned in passing that the compression pulse of the bare femoral artery of the dog recorded by a Frank capsule on a rapidly moving surface shows the effect of this passage of blood through the artery, and that it becomes obvious, as does the passage of blood, at compressing pressures about 10 mm. of mercury above the level at which the first sound is heard.

The fact that blood and even a "rudimentary" pulse may begin to pass beneath the compression before the so-called "fully developed" pulse (8,3) has been known for some time. The present experiments prove, however, that the artery first opens by orifices so narrow that the pulse is entirely lost in them. These must be orifices formed by folds in the wall of the collapsed artery, for if the first blood that succeeds in getting through were forced through by the opening of the artery by the pulse, it would show a pulsatile progression from the very first. The later faint pulse may be taken to indicate that these orifices are now large enough to let through some of the pulse. But the abrupt appearance of the brusque pulse indicates the entrance of a wholly new phenomenon. This new phenomenon could be but one thing, namely the separation of the walls of the artery throughout their entire periphery, and not merely where they are creased.

Effect of the size of the compression chamber in animal observations

It is not difficult to show that in observations on animals the compressibility of the compression space and the phase of the pulse cycle in which the compressibility of the space is reduced have consequences quite similar to those seen in the experiments with the circulation schema. As, however, the results obtained in the animal experiments must be considered in detail in connection with the mechanism of sound production in arteries, it will not be necessary to discuss them now.

SUMMARY

Experiments are described which were designed primarily in an effort to harmonize the conflicting views that are held with regard to the significance of the critical pressure oscillations yielded to a space through which an artery is being compressed. At the same time it was hoped that additional information in regard to the mechanism of the compression oscillations might be brought to light.

Experiments have been performed both on rubber tube and artery in a circulation schema operated by a method of procedure described by Brooks and Luckhardt, and on arteries in situ. The results have been as follows:

1. In the case of one and the same tube or artery the general configuration of the record of the compression pulses depends upon (a) the compressibility of the compression space and, if that is sufficiently small, upon (b) the phase of the pulse cycle in which the compression space is closed. The variations in configuration may be so marked under the different conditions as to show maximum oscillations at systolic, diastolic or mean compression pressures. Some of the discrepancies in the views held with regard to the significance of critical oscillations undoubtedly are attributable to the differences in the experimental conditions enumerated above.

2. The configuration of the oscillation record is influenced also by the extensibility of the artery, by the significance of the upper and lower conical closures of the artery relative to that of the completely occluded part between (length of artery), and by the relation of the volume of the undistended bore to that of the distended bore of the artery in the compression chamber.

3. When the compression space is sufficiently large the compression oscillations are proportional to the volume changes of the artery produced by the pulse and to the compressing pressure.

4. The volume change with each pulse is then determined by the difference between the volume of blood in the artery under the compression chamber during diastole and during systole.

5. During decompression the diastolic volume increases constantly though along three successive gradients, each of which is in the main determined by a different process, namely (in the order of their appearance): (a) the descent of the upper conical closure of the artery, (b) the ascent of the lower conical closure, and (c) the filling of the intermediate segment of the artery to its undistended bore (at diastolic

compression) and the subsequent stretching of the arterial walls at compression below diastolic pressure.

6. The systolic volume also increases constantly and along three gradients determined by the following processes respectively: (a) the descent of the upper conical closure, (b) the filling of the central segment to its undistended bore (at systolic compression) and (c) the subsequent stretching of the arterial wall.

7. The diastolic and systolic volume gradients are so related to each other that the compression oscillations determined by their separation in different stages of decompression have the relative amplitudes usually seen in records of the blood pressure made by the oscillatory method; though it is obvious that as a result of differences in the relative significance of the factors determining the systolic and diastolic gradients variations from the typical record must frequently occur. Thus the slight diminution in the amplitude of the oscillations frequently observed before the sudden diminution begins is attributable mainly to an increase in the influence of the lower conical closure of the artery.

8. However under all circumstances, natural as well as artificial, a sudden increase and a sudden decrease in the amplitude of the oscillations, if present, indicate accurately the systolic and diastolic pressures respectively.

9. It is shown that with a pulse of the configuration of the arterial pulse the maximal oscillation must be and is recorded at a time when the artery is still collapsed in the diastolic phase of the pulse cycle by the pressure from without. The maximal oscillation can be obtained at a time when, during decompression the artery has attained the "half-flattened" state (MacWilliam and Melvin) only if the pulse has an atypical form, such as probably could be developed under artificial conditions only.

10. During decompression a slight flow of blood, which soon becomes faintly pulsatile, begins about 10 mm. of mercury above the compressing pressure at which a brusque pulse, undoubtedly marking the first opening out of the artery from the "collapsed" state, appears.

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THE EFFECT OF NICOTINE UPON THE REFLEX ACTION OF SOME CUTANEOUS SENSE ORGANS IN THE FROG

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Received for publication December 18, 1915

The basis of this investigation was to determine the effect of nicotine upon certain skin reflexes in the frog: to determine the duration of this effect; its after effect; if immunity could be established; and how the action of nicotine compares with that of alcohol. The experiments were conducted upon frogs of the species Rana Pipiens, secured from a nearby lake. They weighed from 15 to 120 grams, and during the period of experimentation were kept in a dark room in cool moist moss, without food.

The sensory ending of the cutaneous nerves may be considered peripheral end organs. By carefully testing certain spots, some were found which responded with a fairly constant reflex time, to a definite stimulus. The same spot varies somewhat at different times of the day and also at different room temperatures; on cool days the response was quicker.

The spots selected for study were those which proved after careful testing to be most reliable and constant in their reaction time. These spots are illustrated in text, figure 1, and were the same ones employed in a former experiment (1).

The constant stimulus determined upon, was one that would not injure or fatigue the peripheral nerve endings during the period and method of experimentation. Pure neutral filter paper, three millimeters square, moistened with 8 per cent pure acetic acid was found the most practical chemical stimulus. The paper was moistened in the acid, then placed carefully by means of a long forceps upon the spot to be tested. That the errors due to sight and pressure stimuli were avoided, was proved by control experiments in which the eyes were covered by a special device.

By reflex time is meant the interval between the moment the paper touches the skin and the moment the frog made an attempt to remove it. It was found that if the attempt was not made within one minute, it usually never would be. As soon as an attempt was made or if not made at the end of one minute, the acid paper was washed off with fresh water thus preventing fatigue and injury to the peripheral nerve ending. This was proved by testing the corresponding spot on the opposite side and also by control experiments on frogs which had not been given nicotine.

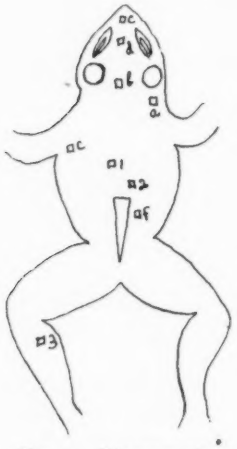

Fig. 1. Diagram showing location of sensory spots on the frog. Those on the head are innervated by branches from the cranial nerves, those on the trunk and legs by spinal nerves.

The nicotine used in these experiments was dissolved in distilled water and injected into the dorsal lymph sac. The doses employed varied from $\frac{1}{2}$ minim of 0.05 per cent to 27 minims of 0.1 per cent nicotine per 10-gram frog. Control experiments were made by injecting Ringer's solution in amounts equal to the maximum amount of fluid introduced with the drug. These control experiments served to check the mechanical effect of injection and the effect of dilution on the circulating fluid.

In carrying out an experiment, the reflex time of the series was found upon the frog before nicotine was given, then the drug was injected, and 10 minutes were allowed to overcome the mechanical effects before testing the spots again. This testing was repeated every ten minutes for about two hours or until the reflex response returned, allowing an interval of 10 minutes rest between each test. If the reflex response did not return at the end of two hours, the tests were repeated the following day. The acid paper was applied with one hand and the stop watch was used with the other to record the reaction time. Philips and Pembrey (2) give 10 minims (0.59 cc.) of 1 part of nicotine in 20 parts saline solution as a toxic dose and Cushny 1 cc. of 0.2 per cent solution. The reflex time and effects secured after injection of nicotine, were compared with the reaction time and effect obtained before injection, also with the reaction time after injection of Ringer's solution.

It was found that each spot had its own reflex time, its own degree

of irritability and that some were more resistant to nicotine than others. Spot C (fig. 1 and Table I) is normally irritable, usually responding to the acid stimulus in one second, and after doses large enough to cause a loss of reflex, was always the first to recover; often being the only spot to give a reaction response. Spots f and c also respond quickly to the acid stimulus and recover quickly from the seemingly paralyzing effect of the nicotine. Spots 1 and e seem to be the least irritable

TABLE I

Comparison of irritability of spots

SPOTS	I REACTION TIME BEFORE NICOTINE	II REACTION TIME AFTER NICOTINE	III LENGTH OF PARALYZING EFFECT		
			0.5 m. (a)	6 m. (b)	14 m. (c)
1	2 seconds	4 seconds	40 minutes	100 minutes	180 minutes
2	12 seconds	20 seconds	40 minutes	100 minutes	180 minutes
3	2 seconds	4 seconds	20 minutes	80 minutes	120 minutes
a	7 seconds	8 seconds	20 minutes	100 minutes	360 minutes
b	8 seconds	38 seconds	40 minutes	150 minutes	360 minutes
c	1 second	1 second	10 minutes	50 minutes	120 minutes
d	5 seconds	5 seconds	20 minutes	50 minutes	120 minutes
e	15 seconds	30 seconds	40 minutes	150 minutes	360 minutes
f	4 seconds	10 seconds	20 minutes	75 minutes	180 minutes

For location of spots, see text, figure 1.

M. Minims, 1 drop, 0.16 cc.

Stimulus-acid paper, see text.

Nicotine of 0.05 per cent.

Column I shows reaction time to an acid stimulus for a normal frog.

Column II shows the immediate depressing effect of nicotine after paralyzing effect has passed off. e. g. Spot I in a normal frog responded to the acid stimulus in 2 seconds after the paralyzing effect, that lasts for 40 minutes, has passed off, its reaction time to the stimulus increased to 4 seconds.

Column III shows the length of time that the paralyzing effect of a weak, medium, and strong dose lasted for the different spots.

and show the least resistance to the action of the drug. It was found that spots e, 3, and f were the most irritable normally and showed the most resistance to the action of the drug. Table I also shows that nicotine has both a paralyzing and a depressing effect. First it paralyzes the ganglia cells (according to Langley), then as soon as the paralyzing effect passes off, there is a depression of the spot, making it slower in its response to the acid stimulus shown in Column II and I. Column III shows the duration of paralysis in the different spots,

due to the doses indicated. For instance spot c lost its reaction time for 120 minutes, while b lost it for 360 minutes.

It was interesting to note that as weak a dose (Table I, III a) of nicotine as ½ minim of 0.05 per cent per 10-gram frog produced a change in the reflex time of the cutaneous sensory spots, but no apparent effect on other reflex actions, such as turn over, compensatory, and swimming, or upon the general behavior of the frog. It had a more depressing effect than a larger dose of Ringer's solution. With this dose, spot c was the only one which did not lose its irritability or fail to respond to the acid stimulus at any time. Small doses from ½ to 5 minims per 10-gram frogs were followed for about 15 minutes by slightly forced or labored breathing, and also by a slight constriction of the pupils. Weak fibrillary twitchings were also noticed immediately after injection. These lasted only for a short time, during which the position was usually normal.

With medium doses (Table I, III, b) the skin reflexes were all lost and for a longer time, the breathing became more forced, the constriction of the pupils more apparent, and the fibrillar twitchings more pronounced. The frog assumed a flattened position, and even after the deepened breathing became normal again, seemed sluggish.

With large doses (Table I, III a) i.e., of 10 minims of 0.05 per cent per 10-gram frog and over, the higher reflexes as well as skin reflexes were lost for 1 hour or longer. It was noticed that when the spots failed to respond after a dose of nicotine, they did so immediately. The paralyzing effect increased with increase of the dose. The frog returned to a seemingly normal state within two days, but the skin reflexes usually displayed an increased irritability for some time. There was a period of inhibition of respiratory movements at first, the length increasing with an increase of dose. With the largest doses, this inhibition lasted for two hours or longer, and was followed by forced irregular breathing.

The large doses also caused an immediate stiffening or tetanus of the front legs, which lasted about 15 minutes. This stiffening spread somewhat to the trunk and hind limbs, then was followed by a relaxation and loss of muscle tone, and continued until the higher reflexes again made their appearance. From these observations we conclude.

1. That nicotine causes a loss of the skin reflexes, a seemingly paralyzing effect, that is followed by one of depression.

2. That nicotine in small doses causes forced breathing; in large doses an inhibition of respiratory movements, followed by forced irregular breathing.

3. That it causes fibrillar twitchings and constriction of the pupils.

4. That large doses cause tetanus contraction of the front legs, and sometimes a slight stiffening of the whole body, followed by relaxation and loss of muscle tonus.

II. TOLERANCE EXPERIMENTS

In connection with the above observations, a series of experiments were carried on to determine if tolerance to nicotine could be established. A number of frogs were given first a minimum dose, ½ minims of 0.05 per cent per 10-gram frog, then at intervals of two days were again injected with doses of one minim until the last dose given proved toxic to a normal frog. To prove that laboratory conditions, and fatigue due to acid tests, were not factors to be considered, control frogs were placed under the same laboratory conditions, and subjected to the acid tests as frequently, and on the same spots, as were those employed in the tolerance experiments.

Table II shows the typical effects of equal doses of nicotine upon a normal frog and upon one in which tolerance is being established, by giving it gradually increasing doses. In comparing the effects of nicotine on these frogs it was seen that with small doses, there was considerable difference in the effect upon the two frogs. The normal one lost all the skin reflexes for a time and exhibited fibrillar contractions. The only apparent effect upon the tolerant frog was the forced breathing and increased irritability. Its skin reflexes all responded to the acid stimulus at the end of 10 minutes. With medium doses 10 minims of 0.05 per cent to 7 minims of 0.1 per cent per 10-gram frog, the most noticeable difference was that, although both frogs passed through the different stages produced by nicotine, they did not last so long in the tolerant frog, and its skin reflexes responded much more quickly to the acid stimulus. It was interesting to see that sometimes the skin reflexes responded even before the higher reflexes returned, this being contrary to the usual events.

Other interesting observations taken on the tolerant frog were, that after recovery from the effect of the drug the animal became very irritable and the spots responded abnormally, all of them within one second. Often the slightest touch on the skin called forth a violent reflex movement. The tolerant frog also began to show a yellow discoloration of the skin on the under side of the lower part of the body and hind legs. Although this discoloration remained permanent, it was most noticeable shortly after injection. It was also found, that there

TABLE II

Comparison of normal and tolerance frogs

FROG	DOSE PER 10 GRAMS	% NICOTINE	SKIN REFLEXES LOST	HIGHER REFLEXES LOST	IRRITABILITY	CONSTRICTION OF PUPILS	POSITION	BEHAVIOR	RESPIRATION
Normal	5 m	0.05	15 min.	No loss	None	Very slight	Normal	Normal	Deeper
Tolerance	5 m	0.05	No loss	No loss	Very slight	Very slight	Normal	Normal	Deeper
Normal	3 m	0.05	32 min.	No loss	None	Slight	Not normal	Slight twitchings	Forced
Tolerance	3 m	0.05	No loss	No loss	Slight	Slight	Not normal	Normal	Forced
Normal	6 m	0.05	More than 2 hrs.	No loss	None	Constricted	Flattened	Twitchings	Forced
Tolerance	6 m	0.05	No loss	No loss	Quiet	Constricted	Slightly flattened	Normal	Forced
Normal	10 m	0.05	6½ hours	60 min.	Sluggish	Very constricted	Very flat	Twitching	Inhibition, followed by irregular
Tolerance	10 m	0.05	83 min.	60 min.	Sluggish	Very constricted	Very flat	No twitching	
Normal	14 m	0.05	More than 12 hrs.	More than 4 hrs.	Sluggish Loss of mus. tonus	Very constricted	Stiffening of front legs	No twitching	Inhibition at first then irregular
Tolerance	14 m	0.05	125 min.	95 min.	Loss of mus. tonus	Very constricted	Stiffening of front legs	No twitching	
Normal	18 m	0.05	More than 12 hrs.	More than 6 hrs.	Not very	Very constricted	Very flat	Twitchings follow injections	Apparent inhibition for some time
Tolerance	18 m	0.05	More than 12 hrs.	More than 6 hrs.	Very irritable	Very constricted	Very flat	Twitchings follow injections	Apparent inhibition for some time
Normal	7 m	0.1	About 12 hrs.	More than 6 hrs.	Not very	Very constricted	Very flat	Twitchings follow injections	Inhibited followed by irregularity
Tolerance	7 m	0.1	Never returned	Never returned	Lost	Very constricted	Very flat	Twitchings follow injections	Never recovered
Normal	9 m	0.1	Never returned	Never returned	Lost*	Very constricted	Very flat	Twitchings follow injections	Never recovered
Tolerance	9 m	0.1	Never returned	Never returned	Lost	Very constricted	Very flat	Twitchings follow injections	Never recovered

was a decided loss of weight of the frogs which were given frequent doses of nicotine, while the control frogs kept under the same conditions showed but slight loss, due perhaps to lack of food. As the toxic dose was reached, the tolerant frog seemed to become weaker and its power of resistance lessened.

Some of my results corroborate those published by Langley (3), who found that in the skate, 1 per cent nicotine has an extraordinarily strong and local effect upon the bulb and cells in the sympathetic ganglia, also that the application of nicotine to the spinal bulb causes for a time, cessation of the respiratory movements in consequence of the tetanic contraction of the muscles; the muscles then relax and feeble respiratory movements occur. He explains the fibrillary twitchings by the facts that nicotine is a stimulant to the motor nerve cells of the bulb and spinal cord. After this stimulation, however, there is a paralyzing effect. I also found as did Cushny (4) and Sollman (5), that nicotine caused a constriction of the pupil.

In comparing the results exhibited by nicotine with those obtained in a previous investigation with alcohol (6), I found that the differences are quite marked. Both cause a loss of the skin reflexes and in sufficient quantities, loss of the higher reflexes, but nicotine is much more powerful. A dose of 0.008 cc. of 0.05 per cent nicotine per 10-gram frog affects the animal more intensely than a dose of 0.005 cc. of 15 per cent to 30 per cent alcohol per 10-gram frog. Nicotine produces a slight stimulation at first, alcohol never stimulates. Alcohol did not affect the respiration or cause constriction of the pupils. But in large doses, it too caused tetanic contraction of the whole body and death. Nicotine causes a peculiarly characteristic tetanus of the front legs followed by a relaxation and loss of muscle tonus.

This investigation was undertaken at the suggestion of Dr. Ida H. Hyde, to whom I am greatly indebted for constant supervision and help.

SUMMARY

1. Certain sensory spots in the frog's skin differ not only in irritability and reflex action, but also in susceptibility to the influence of nicotine.

2. The skin reflexes are affected by much smaller quantities of nicotine than are the higher reflexes, turn-over, compensatory, and swimming.

3. Small doses of nicotine cause a depression or loss of the reflexes, fibrillar contractions, forced respiration and a slight constriction of

the pupils. One dose did not usually show an increased irritability as an after affect. These changes appear immediately after injection, and last from one-half to three or four hours, depending upon the dose.

Doses of $\frac{1}{2}$ minim of 0.05 per cent per 10-gram frog have a greater effect than larger doses of Ringer's solution. Large doses of nicotine cause an entire loss of the skin reflexes, and muscle tone, producing a flattened position; an inhibited then irregular respiration; constriction of the pupils; loss of turn-over, compensatory and equilibrium reflexes; a tetanus or stiffening of the front legs, followed by a relaxation and loss of muscle tonus.

4. Continued gradually increasing doses of nicotine cause tolerance to the drug, such that it loses some of its effect. Continued use of nicotine causes increased irritability of the skin reflexes, making them respond abnormally. It also causes a loss of weight, and a discoloration of the skin. The effect upon respiration and constriction of pupils is the same as in a normal frog, except that the effect does not last so long.

5. The toxic dose obtained from the results of a large number of experiments, showed that it varied from 6 to 27 minims of 1 per cent nicotine per 10-gram frogs. Some frogs naturally proved more resistant than others. For those frogs which had been given continued gradually increasing doses, the toxic injection varied from 6 to 9 minims of 1 per cent per 10-gram frog. Nicotine acted as an accumulative drug lessening the bodily resistance until the toxic dose is found to be less for the tolerant frog than for the normal one.

6. Nicotine is much more powerful in its effects on cutaneous reflex reactions, ciliary muscles, respiratory activity and bulbar centers than is alcohol.

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THE COAGULATION OF BLOOD IN THE PLEURAL CAVITY

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Received for publication December 18, 1915

The fact that blood in the pleural cavity remains fluid or partially fluid and that such blood fails to coagulate after withdrawal has been shown experimentally by Penzolt (1), Pagenstecker (2), and more recently by Zahn and Walker (3). It has also been observed clinically that blood in the pleural cavity generally remains fluid or partially so and does not clot when withdrawn.

The experiments of Zahn and Walker show that small amounts of blood (5 to 8 cc.) introduced slowly into the pleural cavity either from the internal mammary artery or by means of a syringe, remain fluid. To obtain this result they lay stress on the fact that small amounts must be injected and that deep artificial respiration must be maintained. Such blood when withdrawn from the pleural cavity some 10 to 20 minutes after injection can not be coagulated by addition of thrombin, calcium or thromboplastic extracts. By the heat test fibrinogen was not detected and it was only by the addition of a fibrinogen solution that the blood could be coagulated. Since these authors were unable to find any fibrin in the pleural cavity either microscopically or macroscopically they concluded that the fibrinogen was in some way altered or destroyed by contact with the pleural endothelium. They were, however, unable to show that extracts of this endothelium had any retarding action on the coagulation of whole blood in the test tube.

We have repeated their experiments on dogs many times using their technique. In our most successful experiments, the blood was found to be almost entirely fluid but in all cases we found a small clot generally situated about the hylus of the lung. When injections of blood were given more rapidly and in larger amounts there resulted a relatively larger clot with a smaller amount of fluid blood.

We found, as did Zahn and Walker, that the fluid portion of the blood gave no precipitate on heating to 60°C. and that it would not clot on

addition of thrombin,[1] calcium[2] or thromboplastic solutions.[3] The addition of fibrinogen[4] caused a rather poor clot to form after some hours.

We also determined the amount of anti-thrombin[5] in the fluid portion of the blood and found no appreciable difference between it and a sample of oxalated plasma taken from the same blood used for injection.

Although no increase in antithrombin was found, serum and Ringer's solutions were introduced into the pleural cavity and allowed to remain 10–30 minutes with the hope that they might acquire some anti-coagulating power from the pleural surfaces. This hope was not realized. When tested on whole blood both solutions hastened coagulation in all instances.

The fact that Ringer's solution accelerated coagulation after being in the pleural cavity was probably due to admixture with small amount of serum or pleural exudate containing thromboplastic material. In almost all instances we recovered more fluid from the pleural cavity than was injected. Some of this excess was probably serum or pleural exudate containing thromboplastic material, which would accelerate coagulation when added to whole blood.

As is mentioned by Lord (4), the question has been raised as to whether the failure of these bloods to coagulate is due to a destruction or alteration of fibrinogen by the pleura or to a previous coagulation and defibrination in the pleural cavity.

In order to determine whether fibrinogen alone is altered or destroyed by contact with pleural surfaces the following experiments were made.

FIBRINOGEN INJECTION

Injections of 10 to 30 cc. of fibrinogen (prepared according to a modification of Hammarsten in method) were made into the pleural cavities of dogs, seven such experiments being done. The usual precautions of slow injection and deep artificial respiration were observed and the solutions were allowed to remain in the chest 10 to 30 minutes.

In one instance a small membraneous clot was found on opening the chest but in the other six the fluid was clear or slightly blood tinged.

[1] Thrombin prepared according to Howell's method. This Journal, 1913, xxxii, 264.

[2] 0.5 per cent solution of delequesent $CaCl_2$.

[3] Kephalin or fresh spleen extract. See Howell, This Journal, 1912, xxxi, 1.

[4] Prepared by a modification of Hamarsten method.

[5] Howell's method, Arch. Int. Med., 1914, xiii, 76.

In all cases the fibrinogen after withdrawal was clotted much more rapidly by thrombin than control specimens of fibrinogen which had not been injected.

It has already been pointed out, in speaking of the injection of Ringer's solution, that serum or thromboplastic material is probably responsible for this accelerated coagulation. That thrombin was added to the fibrinogen solutions while in the pleural cavity seems likely since if the fibrinogen were allowed to stand some hours after withdrawal a faint veil like clot sometimes formed indicating that a small amount of thrombin was present.

From these experiments it is evident that fibrinogen itself is in no way altered so far as its power to clot with thrombin is concerned by contact with pleural surfaces. In further experiments an attempt was made to determine what occurs when fibrinogen and thrombin are injected together into the pleural cavity.

INJECTION OF THROMBIN AND FIBRINOGEN

Experiment i. 20 cc. of a fibrinogen solution was injected into the pleural cavity and allowed to remain for about 10 minutes. At the end of this time 5 cc. of a thrombin solution was injected and 20 minutes later the chest was opened a large firm clot being found. The proportion of thrombin to fibrinogen in this case was very large, so much so, that the mixture clotted solidly in the test tube in less than 2 minutes.

Experiment ii. Thrombin and fibrinogen were mixed outside the body and 8 cc. of the mixture was immediately injected slowly into the pleural cavity. The proportion of thrombin was much smaller than in Experiment i, a specimen clotting in 10 minutes in the test tube.

After 30 minutes the chest was opened and 14 cc. of fluid was withdrawn. A long thin clot was found back of the root of the lung but the proportion of clot to fluid was small. The fluid portion did not clot on standing. It was not clotted by addition of thrombin and showed no precipitate on heating to 60°C., indicating an absence of fibrinogen. Adding thromboplastin and calcium failed to cause coagulation but the addition of fibrinogen caused a poor clot to form after some hours.

Experiment iii was identical with Experiment ii except that the proportion of thrombin to fibrinogen was even smaller, a specimen clotting in the test tube in 14 minutes. Immediately after mixing 4½ cc. of this thrombin and fibrinogen were introduced very slowly into the pleural cavity and at the end of 30 minutes the chest was opened and 4½ cc. of

fluid withdrawn. No trace of any clot could be seen but no microscopic examination for fibrin was made.

This fluid remained unclotted for several days and could not be coagulated by thrombin, calcium or thromboplastin. Fibrinogen was absent by the heat test and addition of fibrinogen caused the usual slow coagulation. Addition of fibrinogen and thromboplastin caused a clot in 15 minutes.

These experiments with artificial solutions quite parallel those with the whole blood.

SUMMARY AND CONCLUSIONS

Small amounts of blood introduced slowly into the pleural cavity when deep artificial respiration is maintained will remain in large part fluid. Small clots are always found in the pleural cavity, the size depending on the amount of blood injected and the rapidity of injection.

The fluid portion of the blood can only be clotted by addition of fibrinogen. Thrombin, calcium and thromboplastin are incapable of causing coagulation. The blood shows absence of fibrinogen which perhaps may be removed in some way other than by coagulation.

Pure fibrinogen solution which has been in the pleural cavity under the same conditions, not only is not altered, but clots more readily than the control on adding suitable amounts of thrombin.

Small amounts of thrombin and fibrinogen when mixed in suitable proportions and injected slowly into the pleural cavity remain fluid and show an absence of fibrinogen.

Since it has been shown that fibrinogen alone loses none of its properties after remaining in the pleural cavity, and that the presence of thrombin under the same conditions causes a disappearance of fibrinogen, we can only conclude that coagulation has taken place.

The experiments with artificial solutions exactly parallel those with whole blood, the conclusion being that blood which has been in the pleural cavity remains fluid not because of any alteration of the elements, but because of previous coagulation and defibrination.

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GASTRO-INTESTINAL STUDIES

XII. Direct Evidence of Duodenal Regurgitation and its Influence Upon the Chemistry and Function of the Normal Human Stomach[1]

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Received for publication January 3, 1916

INTRODUCTION

In the course of the extensive gastric investigations undertaken in this laboratory it has long been noted that following the introduction of certain substances into the stomach, the color of the samples removed varied from that of the material introduced to a golden yellow or a dark olive or blue green. The color in the gastric contents is said by Sartory (1) to be sometimes due to the cryptococcus salmonius, and in certain cases of hyperacidity the blue-green mould penicillium crustaceum has been found. But all our samples showing color, on standing exposed to light for one hour became deep green and the oxidation tests for bile pigments were positive. Furthermore, in repeated experiments on the same individual with the introduction of such substances as water, sodium chloride, alkaline or acid solutions, color changes were almost invariably present; while similar experiments with certain other substances as an Ewald test meal, cereals, etc., showed no coloring of the samples. If the color were due to the cryptococcus salmonius or the penicillium crustaceum its presence should be independent of the material introduced. We, therefore, attributed the color changes in these cases to the presence of bile.

By this evidence of regurgitation of duodenal contents we were led to further investigate the theory propounded by Boldyreff (2, 5) "The self-regulation of the acidity of the contents of the stomach." This theory states that the initial high acidity of the gastric juice, namely, 0.32 per cent to 0.48 per cent HCl as has been proven by recent inves-

[1] Reported before American Physiological Society, by title, December, 1914, and read before American Philosophical Society, April, 1915.

tigators (6, 15), is lowered to the optimum acidity of 0.15 to 0.2 per cent HCl by "an influx of intestinal juices into the stomach with the aim of neutralizing the superfluous acid in it. A portion of the strong acid fluid passing from the stomach into the intestines provokes an abundant secretion chiefly of the pancreatic juice, and, if there is not sufficient pancreatic juice, there are also bile and intestinal juice secreted. The acid fluid, moreover, irritates the intestines, thus provoking antiperistalsis which drives the alkaline secretions of the intestines into the stomach until a sufficient amount accumulates which is capable of lowering the acidity of its contents to the usual level of 0.15 per cent HCl." Migai (16) and Cathcart (17) confirmed the theory and recently Milosorov (18), by work on dogs provided with successive intestinal fistulas, observed that the farther from the pylorus the fistula through which the intestinal contents are removed, the lower the acidity of the gastric contents. Carlson (18a) in speaking of Boldyreff's theory says "I am satisfied from my observations on Mr V., that Boldyreff's view is essentially correct." No data were reported. Hicks and Visher (18b) from observations made under Professor Carlson's direction recently report that "In man, an average of 32.6 cc., of gastric juice (acidity 0.411 per cent) accumulated in twenty minutes chewing of food, causing regurgitation in 40 per cent of ten cases, whereas 100 cc. of 0.4 per cent HCl retained in the stomach for twenty minutes caused no regurgitation in 100 per cent of ten other cases." It is worthy of note that bile was taken as the indicator of regurgitation in the above experiments. Hicks and Visher state that "duodenal regurgitation is not the factor of greatest importance in the reduction of the high acidity of the stomach contents."

If duodenal regurgitation does occur we should be able to recognize some of the constituents of the duodenal secretions in the material removed from the stomach. We have already noted the presence of bile in the specimens of gastric contents, but its occurrence being inconstant renders it rather unserviceable as an indicator of the degree of duodenal regurgitation. Then too, pancreatic amylase, amylopsin, could not readily be distinguished from salivary amylase, ptyalin, and pancreatic lipase, steapsin, could not be taken, for a gastric lipase has been said to be present (19). Trypsin because of its characteristic property of digesting protein in alkaline media and the readiness with which it may be determined quantitatively should prove the ideal indicator. We, therefore, undertook the quantitative estimation of trypsin in the samples obtained by fractional examination of the gastric

contents in an effort to determine if possible the relation of duodenal regurgitation to the chemistry and function of the stomach.

Ehrenreich (20) reports a most interesting series of investigations on pathologic cases made by the fractional method of gastric study with test breakfast and milk and egg diets, the samples being extracted by means of a long thin soft rubber Nélaton catheter. He shows trypsin to be present in 37 out of 61 cases and calls attention to regurgitation of duodenal contents as a normal process, but states that his results, not being uniform, do not justify an unqualified confirmation of Boldyreff's theory.

METHODS

The samples of gastric contents were obtained by the usual technique employed in this laboratory, namely, by fractional removal (21) through the Rehfuss tube (22). The experiments were all carried out on normal individuals whose last meal was that of the previous evening. The tube was introduced and the residuum carefully removed for study. Thereupon the material under investigation was introduced and samples of 5 cc. of gastric contents were removed for study at intervals of ten minutes; the experiments being continued until the stomach was empty and further specimens unobtainable.

Studies were made after the introduction of (1) acid solutions, HCl 0.542 and 0.4 per cent, vinegar (acetic acid 2.72 per cent); (2) water; (3) sodium bicarbonate solutions in various strengths 5 per cent, 2 per cent, 1 per cent, 0.65 per cent; (4) small Ewald meal—1 slice of toast and 240 cc. of water; (5) small Ewald meal with 240 cc. of a 1 per cent sodium bicarbonate solution instead of water.

The total acidity was determined by titrating 1 cc. of the sample of gastric contents against $\frac{N}{100}$ KOH, using phenolphthalein as an indicator, the values being expressed as the number of cubic centimeters of $\frac{N}{10}$ KOH necessary to neutralize 100 cc. of gastric contents. Free acidity was determined by the Sahli iodine method (23) and the values similarly expressed.

Alkalinity in certain specimens of a series was determined by titrating 1 cc. of the sample against $\frac{N}{100}$ H_2SO_4 using methyl orange as the indicator and the values expressed as the number of cubic centimeters of $\frac{N}{10}$ H_2SO_4 necessary to neutralize 100 cc. of gastric contents. Upon evidence of acidity in the series the total and free acid were estimated as above mentioned.

Tryptic estimations were made on samples immediately after removal by a method modified by one of us (24).

EXPERIMENTAL

The question of the residuum found in the fasting stomach has been studied most carefully in this laboratory (25, 26). Of thirty-four residua examined for trypsin only two failed to evidence its presence. Charts I and II show the tryptic values to be high in residua of low acidity and low in residua of high acidity. The tryptic values as

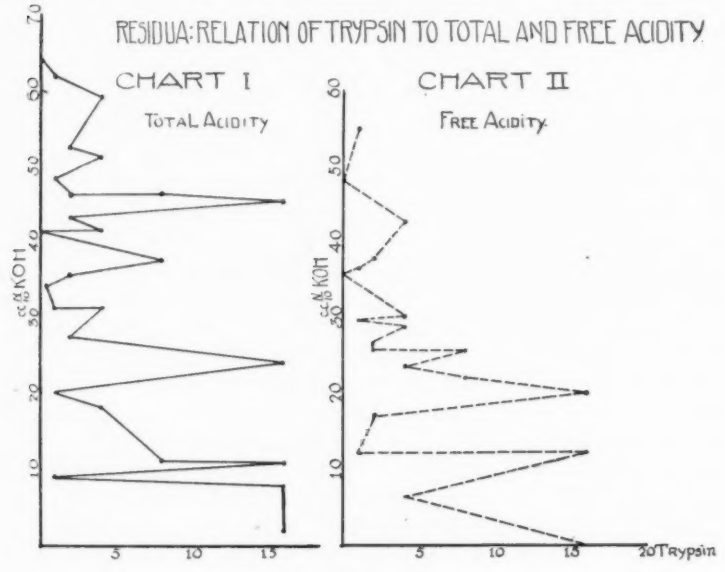
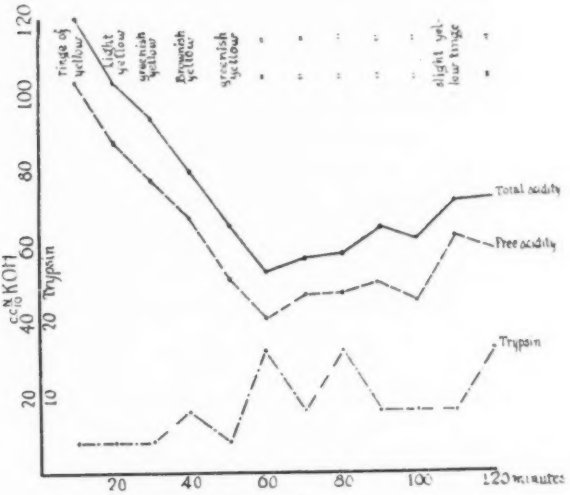


Fig. 1.

plotted in Chart II are shown to be almost inversely proportional to the free acidity. The residua were almost invariably highly colored.

From a total of over fifty experiments we have selected the following as typifying the results obtained after the introduction of the various materials mentioned.

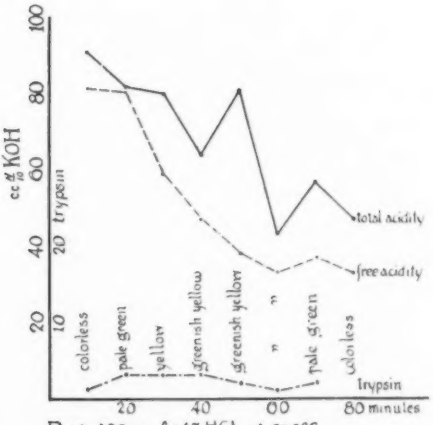
The influence of acid. Chart III is an experiment in which 100 cc. of 0.542 per cent HCl was introduced into the stomach. It is seen that the high acidity is rapidly reduced and with this reduction there is a coincident rise in tryptic values. Following the neutralization of the foreign acid to 0.2 per cent HCl a rise in acidity due to secondary



Diet: 100cc of 0.542% HCl at 23°C.

CHART III

Fig. 2.



Diet: 100cc of 0.4% HCl at 20.5°C

CHART IV

Fig. 3.

stimulation of gastric secretion occurs. The first sample removed was slightly tinged with yellow and as the experiment proceeded the samples became a greenish and brownish yellow. On standing all the specimens assumed a dark green color. Chart IV is the curve of a similar experiment with the introduction of 100 cc. of 0.4 per cent HCl. The fall in acidity is accompanied by a rise in tryptic values, and a marked coloring of the samples substantiated regurgitation. The trypsin curve assumed low values, however. Other experiments with HCl and vinegar (2.72 per cent acetic acid) showed rapid reduction of acidity and corresponding evidences of regurgitation and neutralization. Toward the end of all the experiments the specimens became viscid in character.

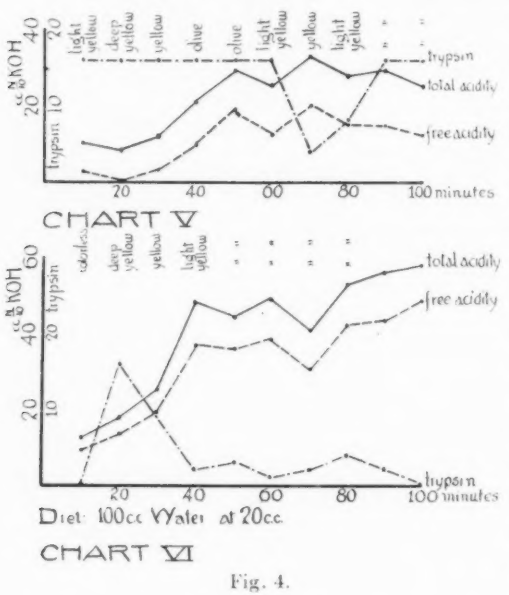


Fig. 4.

The influence of water. Chart V shows a moderate stimulation of gastric secretion following the introduction of 100 cc. of water. The tryptic values are seen to be high, and it is interesting to note that the rather low curve of total acidity reaches its maximum only at that point where the trypsin curve shows its minimum value. This suggests a restraining influence on the acid rise exerted by the alkaline regurgitation in the early part of the experiment. The samples in this case were all highly colored. 100 cc. of water in the subject represented in Chart VI is followed by a more marked stimulation of gastric secretion. In contrast to the previous case the tryptic values run low, although trypsin is present in all specimens. Color changes here parallel the tryptic values.

The influence of a small Ewald meal. These tests were made with

one slice of toast and 240 cc. of water. The tube was introduced, residuum removed, the toast chewed finely and swallowed with the tube in situ, after the ingestion of the toast the liquid was introduced through the tube. Chart VII illustrates a response to the stimulation in a high acidity type of individual. Each specimen shows the presence of trypsin but the curve of the tryptic values takes a low level. No color changes were noted in the experiment. Chart VIII is the experiment repeated on an individual of the low acidity type. Trypsin

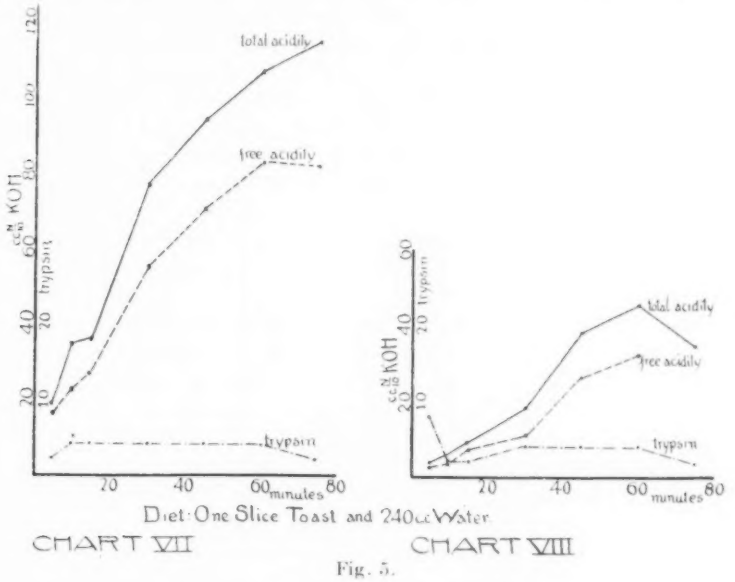


Fig. 5.

is seen to be constantly present, and, as in the previous case, no color changes in the samples resulted. It will be noted that 75 minutes were required for the stomach to empty itself in both of these cases.

The influence of a small Ewald meal with 240 cc. of 1 per cent sodium bicarbonate. Chart IX is an experiment on the same high acidity individual represented in Chart VII. The diet here consisted of one slice of toast but with 240 cc. of a 1 per cent sodium bicarbonate solution substituted for the 240 cc. of water of the previous experiment. The first ten-minute sample showed but slight alkalinity and in the following few minutes the gastric contents became acid, at the thirty-

minute period reaching an acidity of 112 cc. $\frac{N}{10}$ KOH, or over 0.4 per cent HCl. There are two explanations for this fact; one, that an outpouring of gastric juice neutralized the alkali present, the other that the greater portion of the alkaline fluid left the stomach and the remainder was neutralized. The latter seems the more probable, since to completely neutralize the 240 cc. of 1 per cent sodium bicarbonate solution would have required the secretion within a short period of

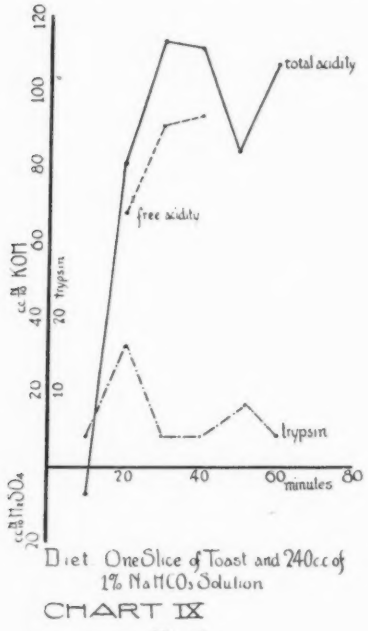


Fig. 6.

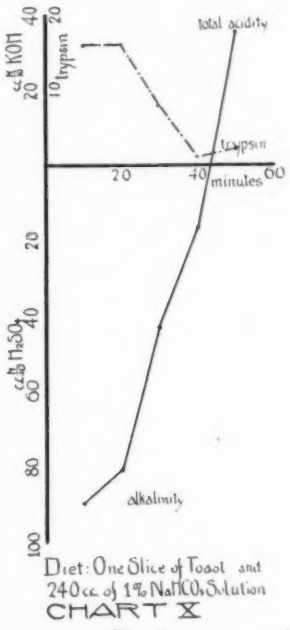


Fig. 7.

200 cc. of gastric juice having an acidity of 0.5 per cent HCl. The fact that the stomach was empty in sixty minutes instead of seventy-five minutes, as with the same diet and water (Chart VII), also favors the latter explanation. This is not in accord with Cannon's theory (27) that the pylorus requires free acid on its gastric side for its opening, since material must have left the stomach while its contents were alkaline in reaction. The high acid values also speak against the theory that sodium bicarbonate inhibits the secretion of gastric juice as stated

by Cannon (28) and Pavlov (29). The presence of trypsin is to be noted in each sample although no evidence of color was found throughout the experiment. This experiment was repeated on the individual represented in Chart VIII. One slice of toast and 240 cc. of 1 per cent sodium bicarbonate solution were given (Chart X). A slower neutralization is shown than in the previous case, as would be expected from the low acidity type of individual (compare with Chart VIII). The same evidence of rapid evacuation is, however, present as in the previous case; the stomach emptying in fifty minutes with 1 per cent sodium bicarbonate solution and toast in contrast to seventy-five minutes with water and toast. It is evident that emptying must have been going on with the gastric contents alkaline in reaction. The tryptic values are high during the alkalinity, thus proving that regurgitation through an open pylorus had occurred. The fall in tryptic values suggests a closing of the pylorus produced either by a direct pyloric stimulus (30), or a duodenal reflex, due first to the coarse food particles and later to an outflow of acid into the duodenun. As in all our experiments with toast and water or alkalies, no color changes occurred in the specimens.

The influence of sodium bicarbonate. 100 cc. of a 5 per cent solution of sodium bicarbonate introduced into the stomach was followed in Chart XI by the neutralization and emptying of the gastric contents in less than forty minutes. A stimulation of gastric juice continues after this time and evidences the truth of the older views that alkalies have some stimulatory influence on gastric secretion. The tryptic values are seen to rise with the acidity and the color changes closely follow the increase in trypsin, suggesting a regurgitation to bring down the rather highly acid secretion to a non-irritating level. The introduction of 100 cc. of a 5 per cent sodium bicarbonate solution (Chart XII) in another case was followed by a slow emptying and a more gradual decline in the alkalinity to about that of the strength of the pancreatic juice. It is to be noted that the stomach emptied while its contents were still alkaline so that no acid was present to open the pylorus. The retention of the solution for ninety minutes indicates that the pylorus was tonic. The tryptic values remained constantly high and most of the specimens contained traces of bile, therefore, the pylorus must have opened intermittently to allow this regurgitation.

Charts XIII and XIV typify experiments in which 100 cc. of a 2 per cent solution of sodium bicarbonate were used in each case. Chart XIII shows rapid neutralization and emptying, followed by marked

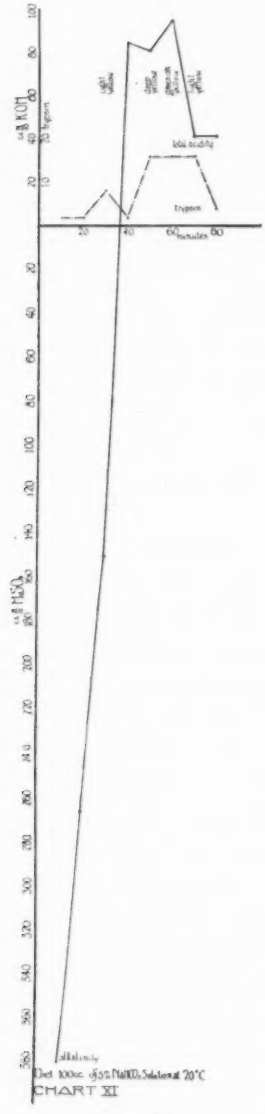

c.c. N/10 KOH
Trypsin
Minutes
c.c. N/10 HCl
Alkalinity
Diet 100cc. 5% NaHCO3 Solution at 20°C
CHART XI

Fig. 8.

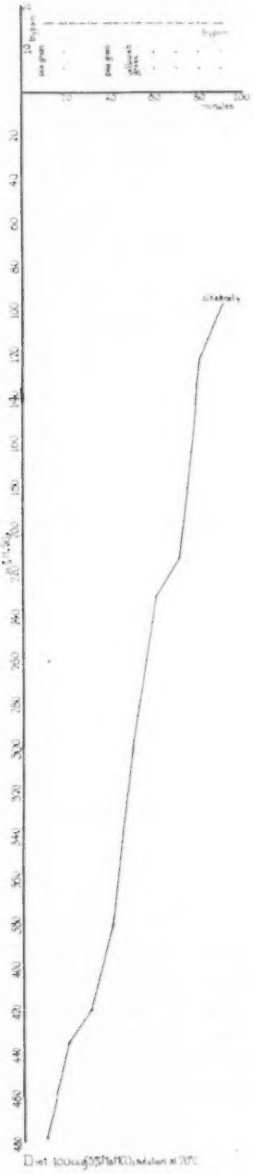


Fig. 9.

stimulation of gastric secretion. A rise in trypsin values and coincident color changes accompany the final fall in acidity. Chart XIV shows the first ten minute sample to be acid and a curious relation of trypsin and color to acidity. The fall in trypsin in the twenty-minute sample seems to allow a rise in acidity, while as the trypsin rises and the color deepens in the thirty-minute specimen the acidity is seen to fall.

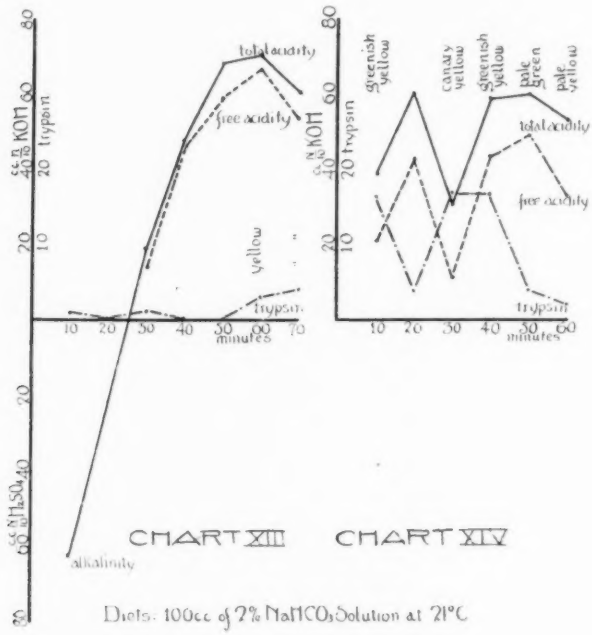


Fig. 10.

Charts XV and XVI are experiments in which 100 cc. of a 1 per cent sodium bicarbonate solution were introduced into the stomach. Chart XV shows rapid emptying of the stomach with alkaline reaction and no gastric secretory stimulation. Trypsin was present in both samples. Chart XVI shows rapid emptying and a marked stimulation of gastric secretion, the first sample being acid. The pronounced tryptic values and high color of the specimens indicate regurgitation of duodenal contents.

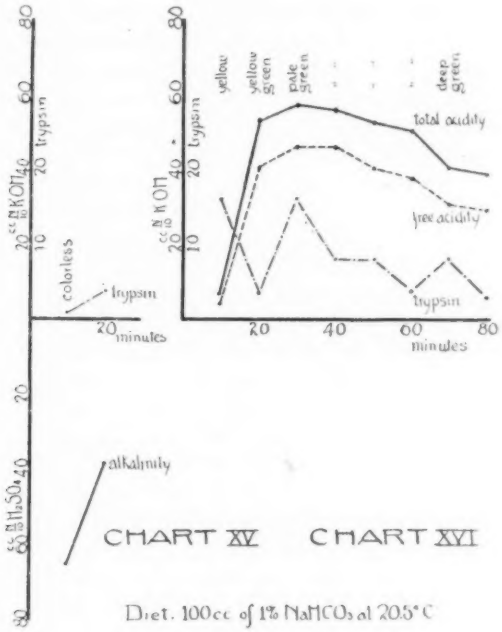
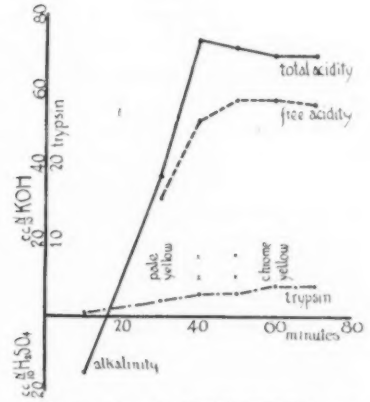


CHART XV CHART XVI

Diet. 100cc of 1% $NaHCO_3$ at 20.5° C

Fig. 11.



Diet: 100c.c. of 0.65% $NaHCO_3$ solution, at 21.5° C.

CHART XVII

Fig. 12.

The introduction of 100 cc. of 0.65 per cent sodium bicarbonate solution, which is said to be the alkalinity of the pancreatic juice (31), was followed by pronounced stimulation of gastric secretion (Chart XVII). Tryptic values rise and the color of the samples deepen as the acidity increases, again suggesting a regurgitation of duodenal contents in an effort to check the mounting acidity.

DISCUSSION

One of the most striking facts to be observed in these experiments is the almost constant presence of trypsin in the fasting and digesting contents of the normal human stomach. The components of the pancreatic juice have been observed in the stomach of the dog by Pavlov, Schittenhelm (32) and Walter (33). Boldyreff (34) noted their presence when the gastric juice was highly acid. Volhard (35) and Lintvareff (36) found them when fats were in the stomach. Ehrenreich's previously mentioned treatise is the most complete series of the study of trypsin in the gastric contents of patients with gastro-intestinal disorders that we have found in the literature. He shows a tryptic enzyme to be present in a little over fifty per cent of his cases. Bickel (37) noted duodenal regurgitation in a girl with gastric and esophageal fistulae. Ehrmann and Lederer (38) have shown considerable regurgitation to occur in the usual Ewald-Boas test meal. Krivlov (39) reports that 63 per cent of 51 gastric analyses on 32 patients with gastro-intestinal disorders gave positive tests for trypsin. Azzovarisco (40) calls attention to the diagnostic value of trypsin in the gastric contents—it being absent in seven cases with disorders involving the pancreas and its ducts. We must assume from this evidence that the influx of duodenal juices into the stomach is a part of the normal sequence of digestive processes. Sokolov (41) observed that both pancreatic juice and bile when introduced into the larger stomach of Pavlov dogs caused a secretion of gastric juice in the smaller pouch.

It was noted throughout our experiments that normal individuals of the high acidity type usually yielded low trypsin values, while in those of the low acidity type the tryptic power was marked. This is best illustrated by the composite Charts I and II, for the acidity of the residua has been found in this laboratory(42) to be an index of the type of secretion poured out during digestion. This suggested to us the possibility of tryptic digestion occurring in part in the stomach as a sort of compensatory action in cases of low acid and pepsin secretion.

Chittenden and Cummins (43) state that trypsin is active in a neutral or weak combined acid medium.

The introduction of acids into the stomach is followed by a rapid reduction of acidity to about 0.2 per cent HCl or less. The fall of acidity is accompanied by a rise in tryptic values and the presence of bile. The relation between trypsin and acid values may be assumed to be analogous to that found in hyperacidity cases and like them usually shows the fluctuation of trypsin at low values. These low trypsin values may be explained by the character of pancreatic juice, as produced by an acid stimulus: for Pavlov (44) has shown that while the juice secreted under the influence of nerve stimulation is rich in enzymes and protein but poor in alkalies, that produced by an acid stimulus contains relatively little enzymic power or protein but is rich in alkali.

It has been demonstrated (45) that trypsin will adsorb to specific and non-specific substrata, and that this adsorption is dependent upon the H-ion concentration of the solution. When true aqueous solutions or water alone are introduced into the stomach, the low tryptic values with high acidity can hardly be due to adsorption of some of the trypsin, although with the test meal such adsorption is probable.

With regard to the action of the HCl and pepsin of the gastric juice on trypsin. Our experiments were, of course, conducted on freshly removed samples, but we have found trypsin present in samples having an acidity of 110 cc. $\frac{N}{10}$ KOH, which had stood for eighteen hours at room temperature. Other tests have shown that trypsin seems but little influenced by the acid and pepsin in the gastric contents. Long and Muhlman (46), working with artificial laboratory pancreatic products, found that trypsin would withstand an acidity of 0.3 per cent HCl through thirty minutes at 40° C., but the destructive action of HCl on trypsin was much accelerated by the presence of pepsin. Ehrmann and Lederer (47) find trypsin very resistant to the action of the gastric juice. This was confirmed by Ehrenreich (48).

Five per cent sodium bicarbonate solutions, in those cases in which a prompt response of gastric secretion did not result, are seen to be held in the stomach for a considerable period, with marked color changes and high trypsin values in the abstracted samples. This retention seems to be for the purpose of allowing the reduction of the high alkalinity by the acid of the gastric juice. The high trypsin values and color changes may be explained by antiperistalsis and regurgitation, the result of the irritation of the duodenum by the highly alkaline fluid. This irritation in the duodenum also produces retention by closing the

pylorus. In those cases in which the alkaline fluid was of low percentage, or the response of the acid gastric secretion and consequent neutralization to a non-irritating point was prompt, the material quickly left the stomach, and, the rapid flow being directed toward the duodenum, regurgitation was slight and trypsin values consequently low.

It is obvious from the sodium bicarbonate studies that the presence of acidity on the gastric side is not necessary for the opening of the pylorus, as stated by Cannon (49). From our observations we have been led to concur with the European view—that the pylorus is controlled reflexly from the duodenum (50). Acid from the stomach irritates the duodenal mucosa and the pylorus becomes temporarily firmly closed. As this acid is neutralized by the alkaline secretions of the duodenum—the pancreatic juice, bile and succus entericus—the pylorus relaxes and more of the gastric contents are forced into the duodenum. This intermittent opening and closing continues until the stomach is empty (51, 52). Fats as contained in milk and egg white when present in the duodenum are known to close the pylorus (53, 54). Distention of the duodenum also reflexly closes the pylorus (55, 56, 57). Coarse food acting on its gastric side will close the pylorus (58). Liquids at high or low temperatures close the pylorus and are retained in the stomach until brought to body temperature (59). It is claimed that hypotonic and hypertonic solutions also leave the stomach more slowly than isotonic solutions (60, 61).

The evidence seems to demonstrate that materials which are irritating to the duodenum are retained in the stomach in an effort to render them non-injurious to the small intestines. Strong sodium bicarbonate solutions are held until the acid secretion of the stomach reduces their alkalinity to approximately that of the duodenum. Acids in the stomach are partially neutralized by regurgitation of alkaline duodenal contents before they leave the stomach. Non-irritating materials as weak sodium bicarbonate solutions or water leave the stomach rapidly because they do not excite the duodenal reflex necessary to produce the pyloric closure.

We desire to call attention to the stimulatory effect on human gastric secretion of sodium bicarbonate solutions. This fact was first set forth by Claude Bernard (62) and later corroborated by Jaworski (63), Zasjadki (64), Du Mesnil (75), Lenoissier and Lemoine (66) and others. They appear to act differently in animals, for in Cannon's experiments (67) on cats, he noted an inhibition of gastric secretion and a delayed

emptying of the stomach following the mixing of food with 1 per cent sodium bicarbonate solution. Pavlov (68) also states that solutions of sodium bicarbonate varying from 0.05 to 1 per cent inhibited the secretion of gastric juice in dogs. In our experiments on normal human adults we find the reverse to obtain—a marked stimulation of acidity in most cases and evidences of material leaving the stomach during the period in which its contents are alkaline in reaction, together with a shortening of the time required for complete emptying of the stomach. It appears, therefore, that the beneficial results ensuing from the use of weak sodium bicarbonate solutions in gastric disorders are not alone due to neutralization of excessive acidity, but perhaps more to the fact that they more rapidly empty the stomach, preventing stasis and thus shortening the period of work for the stomach and increasing the length of its periods of rest. Reichmann (69) has shown that sodium bicarbonate has no permanent effect on gastric secretion and ascribed its beneficient action wholly to its power to neutralize the acidity in the stomach.

The presence of bile coloring in the samples, in those experiments in which the diet consisted of substances stimulating biliary secretion, bears a close relation to the tryptic values. In general those samples most deeply colored have high tryptic content. This, however, is not the invariable rule.

The previously discussed variation of the quantity of trypsin in the pancreatic juice, depending on whether produced by nerve or acid stimulus renders trypsin not an infallible indicator of the amount of regurgitation. A comparatively small amount of nerve excited secretion may contain a large amount of the enzyme, while a greater quantity of the thin watery secretion of higher alkalinity caused by acid stimulation may contain but little enzymic power. Still the fact that it is always present renders its estimation the best method at hand for the determination of duodenal regurgitation.

Our work in many ways confirms the theory of Boldyreff "The self-regulation of the acidity of the stomach," in that we find regurgitation of duodenal contents a constant factor in the workings of the normal human stomach. The evidence seems to clearly show that the function of the gastro-duodenal portion of the alimentary tract is to so prepare ingested materials that they shall be non-irritating to and best adapted for absorption by the small intestines. Regurgitation of duodenal contents into the stomach is a response to irritation of the duodenum and part of an attempt to render harmless substances that would have an

injurious effect on the small intestines. This phenomenon occurs not only with high acidity but when the gastric contents are alkaline in reaction and seems to be a constant accompaniment of normal gastric digestion.

CONCLUSIONS

1. A tryptic enzyme is almost constantly present in the fasting and digesting contents of the normal human stomach.

2. The tryptic enzyme is deduced to be trypsin regurgitated from the duodenum.

3. Trypsin in the gastric contents is highly resistant to the action of acid and pepsin.

4. In general—the tryptic value is high in the presence of low acidity and in alkaline reaction, and of low value when the gastric contents are of high acid concentration. A fall in the acidity is usually accompanied by a rise in the tryptic values.

5. The color of the gastric contents often changes during the period of experiment from that of the ingested material to a golden yellow or a dark olive or blue green. This color change is due to regurgitation of bile from the duodenum and is absent on a diet of substances which do not cause the outpouring of bile.

6. The tryptic values in the gastric contents usually rise concomitantly with the color change, although in a non-bile stimulating diet the tryptic value seems independent of the color.

7. Sodium bicarbonate in 5 per cent solution is held in the stomach until sufficient HCl is secreted to bring the alkalinity to a point where it is non-irritating to the duodenum. The retention is accompanied by a high trypsin value—suggesting anti-peristalsis in the duodenum in response to an irritant.

8. Sodium bicarbonate in 1 per cent solution hastens the emptying of the stomach either by increasing the motility of the stomach or opening the pylorus.

9. Sodium bicarbonate solutions do not inhibit human gastric secretion, but seem to have a direct stimulatory effect in some cases.

10. Free HCl seems unnecessary for the opening of the pylorus—for the stomach sometimes empties while its contents are still alkaline.

11. 0.5 per cent HCl ingestion is followed by a rapid fall in acidity to about 0.2 per cent, due to a regurgitation of alkaline duodenal contents, as is indicated by the rise in tryptic values coincident with the fall of the acidity. The acid is then emptied from the stomach.

12. Regurgitation of duodenal contents into the stomach is but another of the protective functions of which the body furnishes so many examples and has for its purpose the defense of the small intestines from irritants.

POSTSCRIPT

After this paper was ready for the press the work of Zaitzeff (Russkiy Vrach, 14, no. 29, 1915) came to our notice. In experiments on five dogs with duodenal or intestinal fistulas he demonstrated the regurgitation of pancreatic juice into the stomach and showed trypsin to be present in the stomach contents.

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LIVER CIRCULATION IN RELATION TO GLYCEMIA

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Received for publication December 30, 1915

While many investigations of liver anemia and circulatory changes have been made, the reports are discordant and widely scattered in the literature. The relative importance of the portal and hepatic circulations to glycemia has been but little emphasized. We (1) have recently attributed peptone hypoglycemia to such changes and therefore think it worth while to restate the investigations on liver circulation and to add some new observations, insofar as they relate to glycemia, under the following headings: Portal obstruction or ligation, Hepatic ligation, Anemia due to the simultaneous interruption of both circulations, Hyperarterialisation and changes due to increased venous blood flow through the organ (reversed Eck fistula).

PORTAL LIGATION

Bernard (2) devised a method which caused slow obliteration of the portal vein and found that the operated animals developed an alimentary glycosuria. Burjenko (3) obliterated the portal vein in 35 animals and observed them from one to 14 months afterward without finding glycosuria. The blood sugar was not determined. Allen (4) could not confirm Bernard's findings and suggests that the facts obtained by Bernard may have been due to indirect injury of the pancreas and not to portal ligation *per se*. In support of this view Gilbert and Chabrol (5), found chronic pancreatitis which involved the islets following such operations. Natus (6) also found inflammatory changes in the pancreas following portal stasis and internists (7) believe that portal stasis and disturbance of the liver circulation may be the cause of inflammatory changes in the pancreas sufficient to cause glycosuria. To avoid the stasis and consequent changes in the pancreas and other organs which follow ligation of the portal vein, various investigators made Eck fistulas and studied the effects. Hedon (8) worked with depancreatised animals and claims to have obtained different results

when the blood of a normal animal was transfused into the portal circulation of the depancreatised animal than when it entered the general circulation before passing through the liver. According to Hedon the internal secretion of the pancreas, to be effective, must enter the portal circulation. If this be true the Eck fistula should produce glycosuria. It is generally held however that it does not and in support of this general opinion Carlson and Drennan (9), also Carlson and Ginsburg (9), found that the glycosuria and the hyperglycemia that follows removal of the pancreas can be prevented in pregnant animals by the internal pancreatic secretion of the young in utero, which does not enter the general circulation through the portal system. Forschbach (10) also showed that pancreatic glycosurias may be prevented by parabiosis, and various authors have shown that pancreatic grafts may prevent or lessen the glycosuria. In none of these cases does the internal secretion of the functionating pancreas enter the general circulation through the portal vein and liver.

De Filippi (11), Hawk (12) and Macleod (13) have studied carbohydrate metabolism after Eck fistulas and have never reported glycosuria. Michaud (14) found that Eck fistula prevented adrenalin glycosuria but when the dog received 100 grams of dextrose per os the blood sugar reacted as in the normal animal, i.e., remains normal or increases within normal limits. Macleod (loc. cit, xxii) also found that clamping the portal for short periods of time did not produce glycosuria. It is in such short periods of time that we should expect changes in the concentration of the blood sugar if glycosuria occurs. In slow obliteration of the vessel, or in operations where the animal recovers, the body in all probability would rapidly adjust itself to such changes. If however the blood circulation through the liver is quickly changed, it should be possible to detect changes in the sugar content in the blood in an hour or less. Such changes may not be seen if we rely on glycosuria as the test because the degree of glycemia must be marked before glycosuria appears. The absence of sugar in the urine therefore does not indicate the absence of glycemic changes.

To test this point we selected a number of healthy dogs. Each was anesthetised with ether, a tracheal cannula inserted and also a cannula in one of the carotids from which to obtain the samples of blood for analysis. The ether was kept constant throughout the experiment. Blood was taken immediately before ligation of the portal and again about an hour afterward. The sugar was determined by the Bertrand method. The results are given in Table I.

TABLE I

Result of ligation of the portal vein on the blood sugar

DOG NO.	PER CENT OF DEXTROSE IN BLOOD		TIME AFTER LIGATION IN MINUTES	INCREASE ACTUAL
	Before ligation	After ligation		
2	0.088	0.153	60	0.065
3	0.054	0.105	60	0.051
5	0.066	0.127	75	0 061
6	0.123	0.192	65	0.069
27	0.080	0.146	55	0.066
28	0.084	0.314	60	0 023
29	0.102	0.128	65	0.026
30	0.116	0.167	32	0.051
Average.....	0.089	0.145	59	0.056

LIGATION OF THE HEPATIC ARTERY

Arthaud and Butte (15) after ligation of the hepatic artery found first-hyperglycemia, probably due to the struggling of the animal or to hemorrhage. They took 100 grams of blood for sugar determination. After a transient hyperglycemia a state of hypoglycemia followed. Tangl and Harley (16) after ligation of the abdominal arteries allowed the animals to come out of the anesthesia and found hypoglycemia. However the animals lived only 5 to 7 hours and were undoubtedly

TABLE II

Results of ligation of the hepatic artery

DOG NO.	RESULTS OF LIGATION OF THE HEPATIC ARTERY		TIME AFTER WHEN DETERMINED	CHANGE IN DEXTROSE
	Sugar before operation	Sugar after operation		
1	0.080	0.130	60	0.050
4	0.116	0.178	60	0.062
8	0.120	0.140	60	0.020
33	0.065	0.080	60	0.015
34	0.095	0.150	60	0.055
35	0.087	0.092	60	0.005
9	0.126	0.113	60	−0.013
26	0.148	0.124	60	−0.043
Average.....	0.105	0.124	60	0.019

shocked and moribund. The results are therefore of doubtful value. In keeping with their results Allen (17) found that ligation of the hepatic artery does not lower the dextrose tolerance and does not render the animal more susceptible to diabetes nor does the ligation itself cause diabetes. Piqûre was still effective after ligation of either the hepatic artery or the portal vein, but it has been known from the time of Bernard that the simultaneous ligation of both prevents it.

To obtain further data on the immediate effects of hepatic ligation a number of experiments were carried out in the same manner as that described for portal ligation. The results are given in Table II.

LIGATION OF BOTH HEPATIC AND PORTAL AND COMPLETE REMOVAL OF THE LIVER

Bock and Hoffman (18) worked with rabbits and found that after ligation of both portal and hepatic circulations the blood sugar entirely disappeared (quoted from Tangl and Harley) Seegen (19) ligated the vena cava and the aorta above the diaphragm and kept the animals living with artificial respiration. The sugar content of the blood was reduced in three experiments (I) from 0.146 per cent to 0.04 per cent in 70 minutes and (II) from 0.136 per cent to 0.067 per cent in 36 minutes and (III) from 0.230 per cent to 0.120 per cent in 60 minutes.

Kaufmann (20) confirmed Seegen's results. Minkowski (21) removed the liver from geese and found that the sugar disappeared from the blood. Schenck (22) and Kausch (23) confirmed the work of Bock and Hoffman, and Minkowski, at least to the extent that the blood sugar is much reduced when the liver vessels are ligated. Pavy and Siau (24) studied the effects of liver ligation and removal and concluded that while ablation of the liver causes a fall of the blood sugar the lowest figure they obtained was 0.044 per cent. They never found the blood free from sugar, hence could not entirely confirm the work of Bock and Hoffmann. Further, although they report only two experiments, ligation of both hepatic and portal did not lower the sugar concentration but instead at the end of their experiments the sugar was 0.152 per cent and 0.254 per cent, or more than is normally present. They state that with the exception of Tangl and Harley, other experimenters have found that without the removal of the liver, or blockage of the cava above it, no fall of the sugar concentration of the blood takes place. They think that ligation below the liver may still allow sugar to pass from the liver to the blood.

We made a number of experiments on the ligation of both portal and hepatic vessels and obtained the results given in Table III. The experiments were carried out in the same way as the preceding:

TABLE III

Portal and hepatic ligation

DOG NO.	PER CENT DEXTROSE BEFORE OPERATION	DEXTROSE AFTER	TIME AFTER	INCREASE OR DECREASE
37	0.057	0.054	60	−0.003
1	0.130	0.180	45	0.050
2	0.153	0.136	75	−0.014
4	0.178	0.170	60	−0.008
5	0.127	0.083	30	−0.044
6	0.173	0.126	30	−0.047
7	0.077	0.090	45	0.013
9	0.113	0.076	35	−0.043
Average..... ..	0.126	0.114		−0.012

HYPERAEMIA

Bernard (25) refers to the belief of Pavy that arterialisation of the blood flow through the liver suffices to cause glycosuria. This agrees with the clinical observation that the livers of many diabetics are more or less hyperemic. Jardet and Niviere (26) state that the direct transfusion of the arterial blood of a rabbit into the mesenteric vein of another gives rise to glycosuria. Lepine (27) however could not confirm this assertion. Arthaud and Butte (28) observed glycosuria after ligation of the splenic and right gastro-epiploic arteries, and believe that this glycosuria is due to an increased blood flow through the liver caused by such ligation. Schiff (29) claims that ligation of the afferent renal veins in the frog, by increasing the blood flow through the liver, produces glycosuria. Langendorff (30) failed to confirm this work. Allen (31) studied the influence of varying the blood flow through the liver and concludes that ligation of the hepatic artery does not render the animal more susceptible to diabetes which one might expect if hypoarterialisation was an important factor in diabetes. In the attempt to increase the blood flow through the liver he anastomosed the splenic artery and vein and removed the spleen. He found this did not cause diabetes. The blood was not examined. There was some polyuria which he thinks was nervous, but at the same time the polyuria without glycosuria might be explained by a sudden loss of the glycogen of the liver. The glycogen content of the liver was not examined to decide

this suggestion. In support of the idea, however, it is known that hyperglycemic states may exhaust the glycogen and end in hypoglycemic states. In keeping with this statement, Lepine (32) says that ligation of the hepatic artery is followed in a few hours by a total disappearance of the liver glycogen. Our results do not suggest such a possibility.

Hyperarterialisation of the liver was obtained by two methods: One by turning the aorta into the portal directly by means of a glass cannula. Second: The vena cava and portal were anastomosed as for an Eck fistula, but instead of ligating the portal the vena cava was tied above the anastomosis—Reversed Eck. The aorta was then turned into the vena cava below the anastomosis. The starred numbers in Table IV were obtained by this technique.

TABLE IV

Hyperarterialisation of the liver

DOG NO.	PER CENT OF BLOOD SUGAR		TIME AFTER WHEN BLOOD ANALYZED	CHANGE IN AMOUNT SUGAR
	Before operation	After		
13	0.064	0.050	45	−0.010
14	0.165	0.186	51	0.021
15	0.156	0.200	15	0.044
16	0.149	0.313	68	0.164
17	0.206	0.465	68	0.259
18*	0.122	0.137	61	0.015
19*	0.306	0.330	20	0.024
12*	0.241	0.220	31	−0.021
Average.....	0.176	0.238		0.062

Hypervenosity was obtained by the reversed Eck fistula alone. The results are given in Table V.

TABLE V

Hypervenosity of the liver

DOG NO.	PER CENT OF BLOOD SUGAR		TIME AFTER WHEN BLOOD ANALYZED	CHANGE IN AMOUNT SUGAR
	Before operation	After		
20	0.162	0.150	37	−0.012
22	0.167	0.136	65	−0.031
23	0.072	0.123	85	0.051
24	0.163	0.164	45	0.001
36	0.142	0.214	60	0.072
Average.....	0.141	0.157		0.016

From our experiments it would seem that ligation of portal and hyperarterialisation are the only means to raise the blood sugar. The other changes are so small as to be attributed to the ether and within the limits of error.

DISCUSSION

In the present work we have confined our investigations to acute changes in the liver circulation. We have done so because acute changes are more likely than chronic changes to influence the blood sugar. The rather rapid adaptive powers of the body tend to obscure the influence of chronic changes and while there may be a direct action on the liver that entails marked changes in the blood sugar, the compensatory changes may conceal them. Again, disturbed liver circulation may cause secondary changes in the pancreas and these in turn may cause marked changes in the blood sugar and so obscure entirely any change due solely to the direct action on the liver.

In the acute changes, however, we are not entirely free from obscuring secondary influences. For: If an anesthetic be used, it, *per se* changes the sugar concentration of the blood. If we decerebrate the animal we can not avoid the difficulty. We have found the sugar change due to decerebration is just as great as that due to ether, and Morita (33) has recently shown that in decerebrate rabbits which were allowed to recover, ether, diuretin pain, etc., cause just as great a change in the blood sugar as in the normal animal. If we worked without any of these porcedures the pain and shock produced would cause still greater changes. Agajn the changes in other organs must to some degree also enter into the mechanism of acute cases. Such changes as congestion of the other abdominal organs, and the extra work on the heart that many operative processes involve, can not be without effect.

One of the most difficult questions of the problem to answer satisfactorily is: Whether after an Eck fistula or its reverse, we get the actual increase of the venous blood flowing in the changed direction that the theory demands. In this region as is well known, the venous pressure is very low, consequently the mechanical obstructions which must be introduced by the roughened edges of the operated vessels, and the fall in the general blood pressure may so lessen the blood current that even less venous blood passes through the new route than before the establishment of the reversed Eck fistula. Again after ligation of the hepatic artery the result may be greater than the actual lessening of the arterial flow, because it seems to us that one of the functions of this

artery is to aid mechanically in advancing the low pressure venous blood through the liver to the heart, also following the ligation of the hepatic artery relatively more venous blood probably returns through the portal system. We can devise no method which will eliminate these possible objections, and where so many variables exist it is clearly impossible to state in mathematical terms the changes in the blood sugar which the modifications of the circulation in each artery or vein may cause.

The tables show that ligation of the portal vein caused a rise in the blood sugar of 0.056 per cent or about 62 per cent of the original value. This may be due mainly to asphyxia although the hepatic artery still carries oxygenated blood to the liver. We are inclined to believe from the slight effect of ligation of this latter vessel that its function is mainly mechanical as stated above and that the portal vein is relatively much more important for all functions of the liver. Hepatic ligation caused a rise in the blood sugar of 0.019 per cent or 18 per cent of the blood sugar before operation—about the same or less change than the ether alone would have caused. This is in keeping with the fact that the hepatic artery may be permanently ligated without noticeable change in the welfare of the animal. Ligation of both portal and hepatic caused an increase of 0.012 per cent or 9 per cent of the original sugar, which is less change than the ether alone might have caused. The results of this are also in agreement with the statements of Pavy (34) and Siau. Hyperarterialisation increased the blood sugar 0.062 per cent or 35 per cent of the original content. The mechanism here is probably a flushing out of the sugar or glycogen content of the liver and partly asphyxial due to pressure. The livers in these cases become swollen though not so markedly as might be expected. Hypervenosity caused a change 0.016 per cent or 11 per cent—a figure which ether alone might readily produce. In recent work with ether anesthesia we found (35) that the average increase of the blood sugar in one hour due to ether alone ranged from 5 to 26 per cent of the original concentration. Two only of the modifications of the liver circulation produce noticeable changes in the blood sugar, and these were not sufficient to cause glycosuria. These were ligation of the portal vein and the direct turning of the aortic blood through the liver. Less radical changes were without important effects. It is perfectly obvious therefore that the greatest conceivable uncomplicated changes in the liver circulation can play but a very unimportant part in glycosuria or diabetes.

The changes produced by any of the methods used are not great.

Ligation of the portal vein causes an increase in the blood sugar while ligation of the hepatic artery causes no increase. Several possible explanations are suggested. First, ligation of the portal and the prevention of its blood from passing through the liver causes an accumulation of dextrose from the gastro-intestinal tract simply because it is not removed by the liver. This explanation is not tenable because the simultaneous ligation of the hepatic artery and portal vein causes a decrease which would not be possible if sugar was accumulating in the venous system from the gastro-intestinal tract.

A second explanation: The liver for some unknown reason does not take up dextrose readily from the arterial blood.

Third: Arterial blood takes up glycogen from the liver more readily than venous blood. Hyperarterialisation therefore causes an hyperglycemia while hypervenosity does not. These facts and the lack of hyperglycemia following hepatic ligation leads us to think that venous blood is not capable of getting through the liver with as much dextrose as arterial blood. This may be due to the venous blood lacking any considerable power to take up dextrose from liver glycogen. This has an important bearing on the opinion held by many that asphyxia is an important factor in causing glycosuria. Could not the hyperglycemia be the result of a reaction to asphyxia, i.e., hyperoxygenation of the blood and this hyperoxygenation be the cause of the glycemia? This would be in harmony with the theory of Henderson and Underhill (36).

From Macleod's (37) work on the influence of CO_2 on glycogenolysis we might expect a greater action *ceteris paribus*—from the venous blood than the arterial. The action of CO_2 however can not be considered specific since Phloridzin has the same effect (38). We have found no change in the diastatic action of the blood caused by the various operative procedures, and do not therefore consider any of the changes due to enzyme action.

Summary Table

	INCREASE CALCULATED BY DIFFERENCE IN PER CENT BEFORE AND AFTER OPERATION	INCREASE EXPRESSED IN PER CENT OF SUGAR CONTENT BEFORE OPERATION
Ligated portal	0.056	62
Ligated hepatic	0.019	18
Portal and hepatic	0.012	9
Hyperarterialisation	0.062	35
Hypervenosity	0.016	11

CONCLUSIONS

1. Ligation of the portal vein caused a considerable hyperglycemia.
2. Ligation of the hepatic artery causes no hyperglycemia.
3. Simultaneous ligation of the portal vein and hepatic artery causes no hyperglycemia.
4. Hypervenosity of the liver causes no hyperglycemia.
5. Hyperarterialisation of the liver causes a significant hyperglycemia.
6. The utmost conceivable uncomplicated change in the circulation of the liver can play but a minor rôle in the production of glycosuria or diabetes.

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CORRECTION

In the December, 1915, issue of this Journal (vol. xxxix, no. 2) a number of the figures accompanying the article by Forbes and Gregg entitled: "Electrical Studies in Mammalian Reflexes. II. The Correlation Between Strength of Stimuli and the Direct and Reflex Nerve Response," were, through a misunderstanding, printed on the wrong kind of paper and failed to show many details which appeared in the original photographs. These figures are, therefore, reproduced for reference.

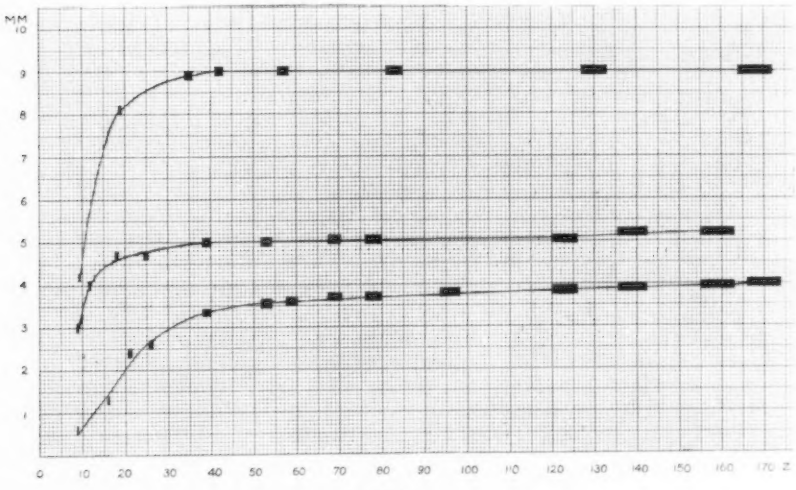


Fig. 1

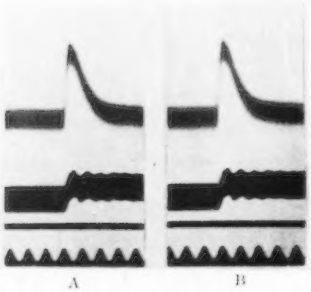


Fig. 2

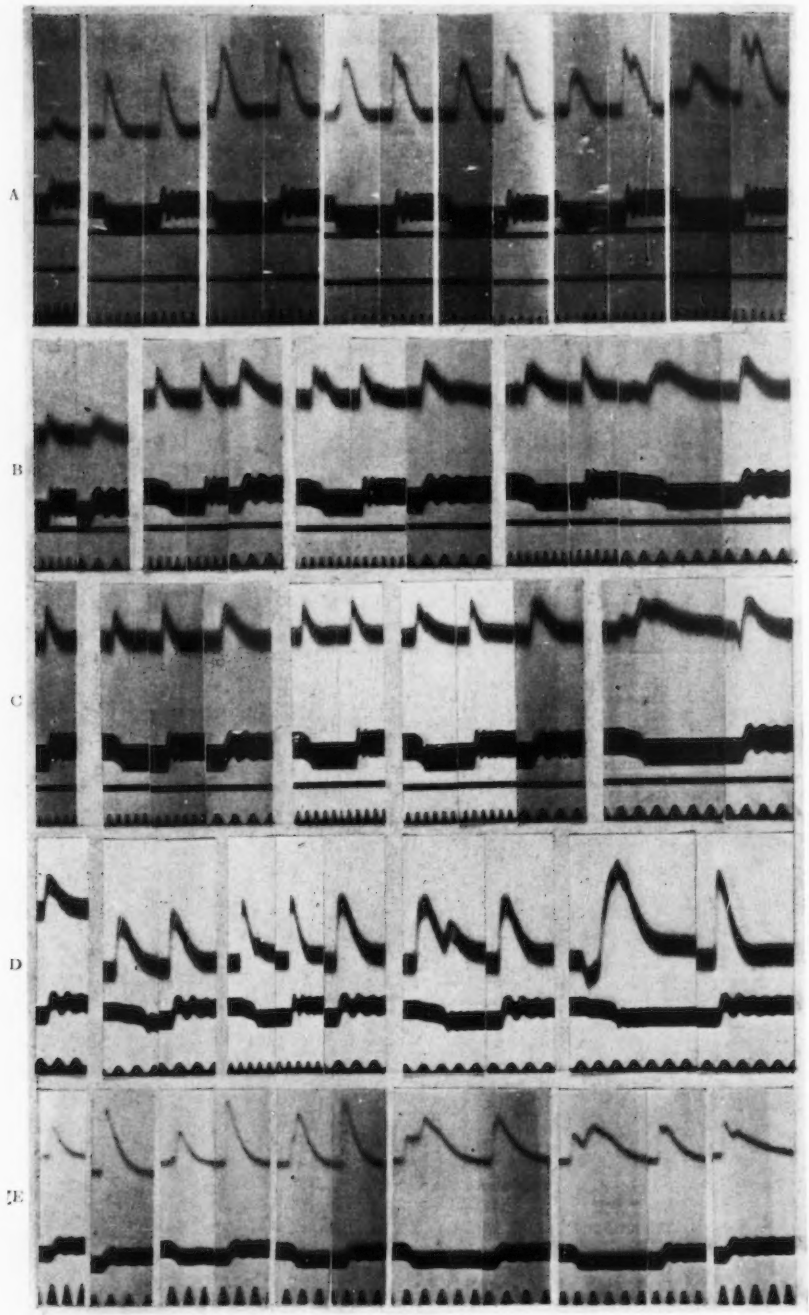


Fig. 3

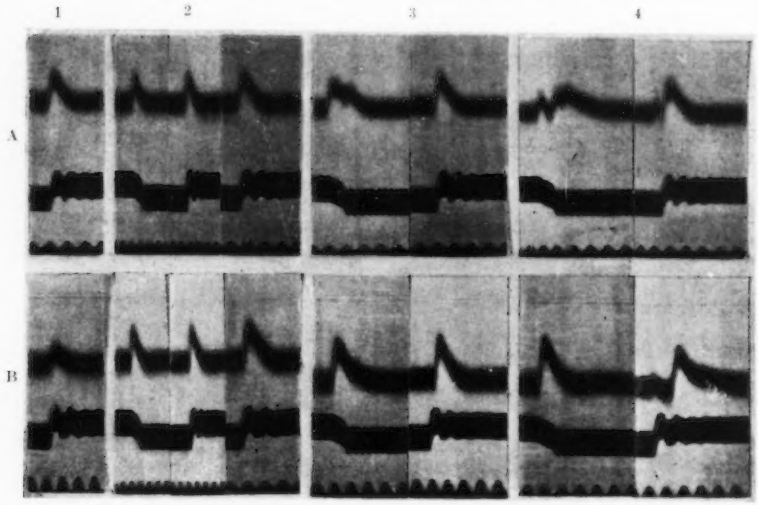


Fig. 4

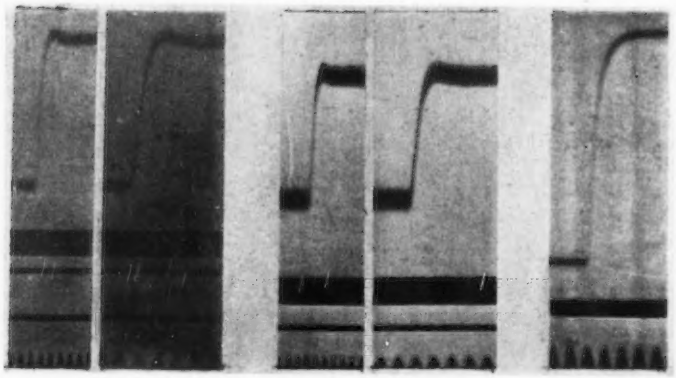


Fig. 5

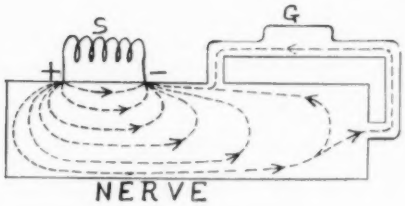


Fig. 6

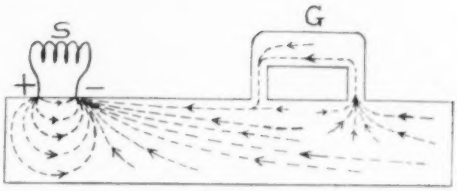


Fig. 7

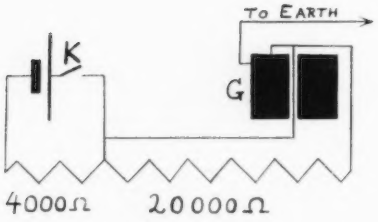


Fig. 8

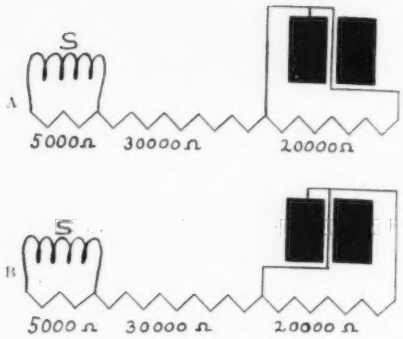


Fig. 10

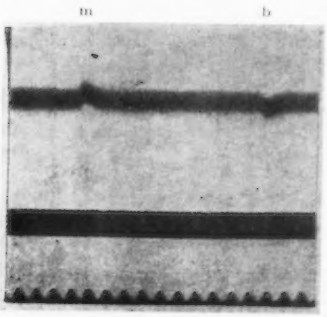


Fig. 9

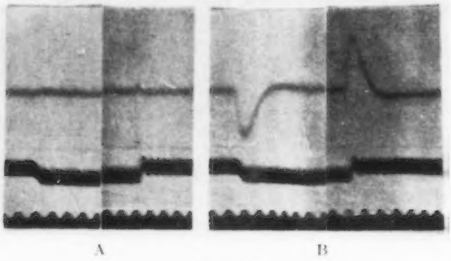


Fig. 12

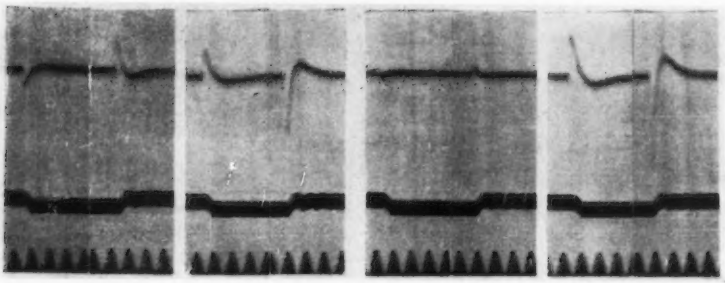


Fig. 11

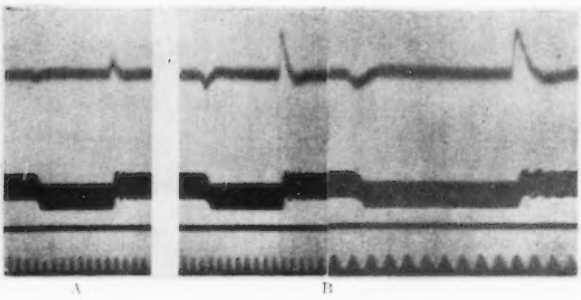


Fig. 13

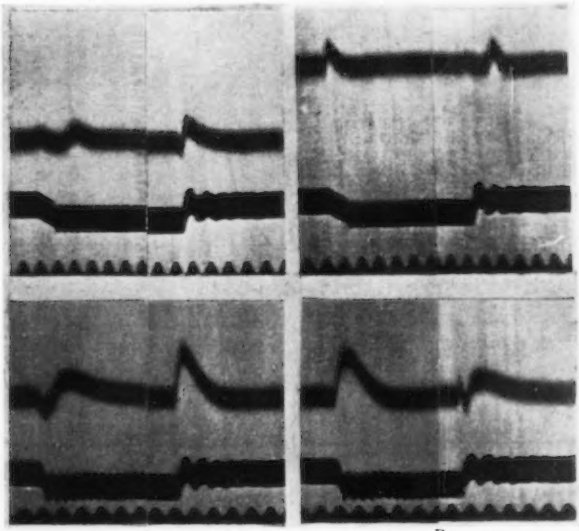


Fig. 15

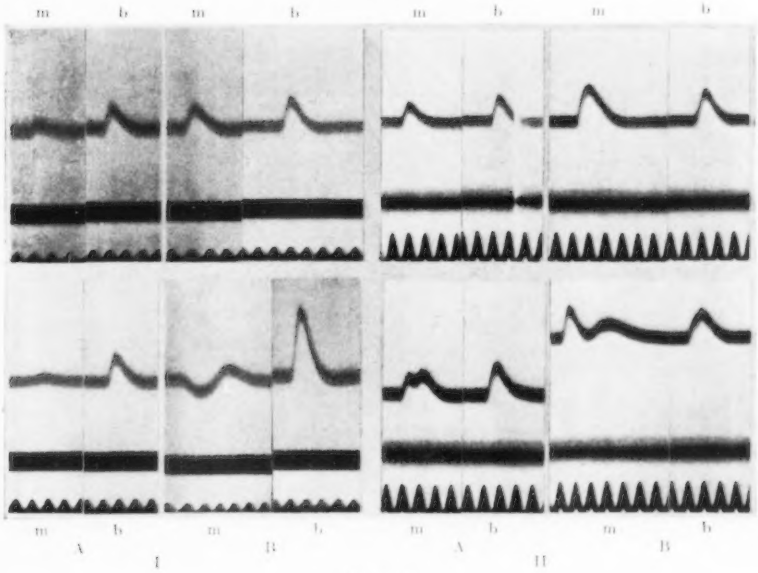


Fig. 16

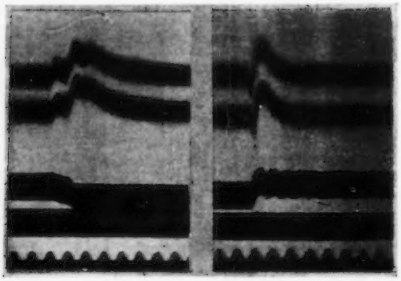


Fig. 14

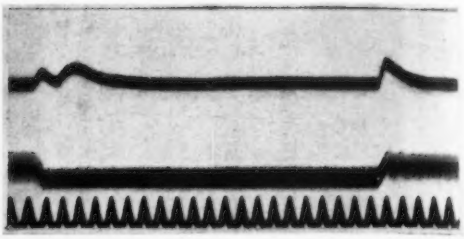
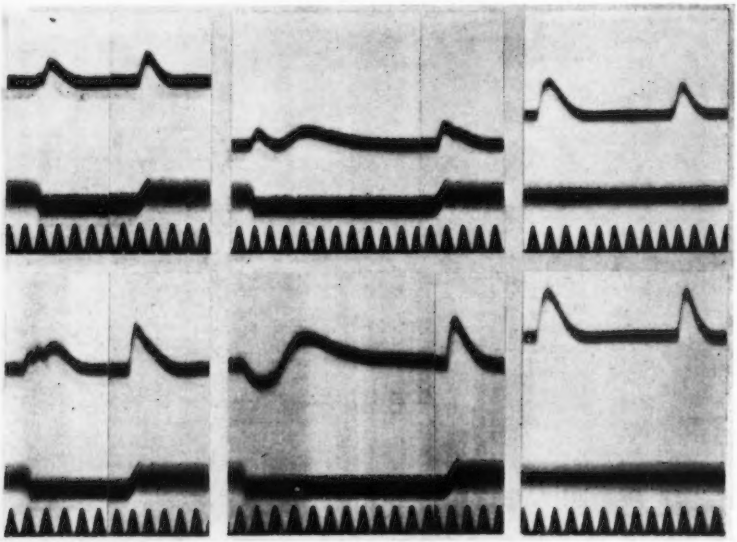


Fig. 17

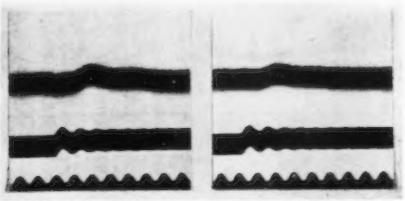


A B C

Fig. 18

A B C

Fig. 19



A B

Fig. 20

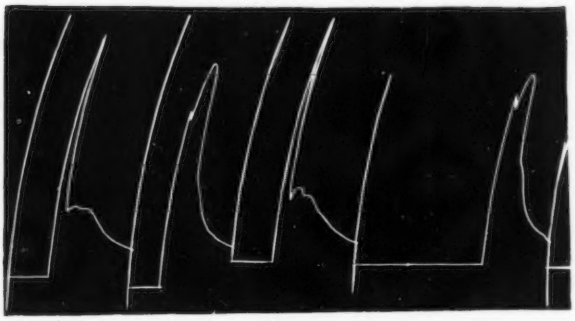


Fig. 21

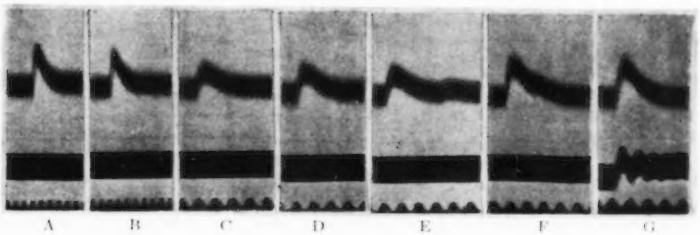


Fig. 22

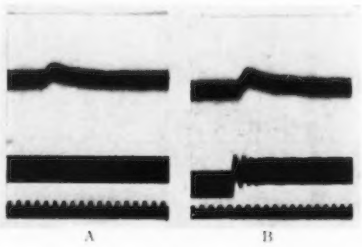


Fig. 23